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## 1940

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# The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

VOL. XIX]

NOVEMBER, 1940

[Nos. 1-3

## TWO NEW ANONACEÆ FROM ASSAM AND BURMA

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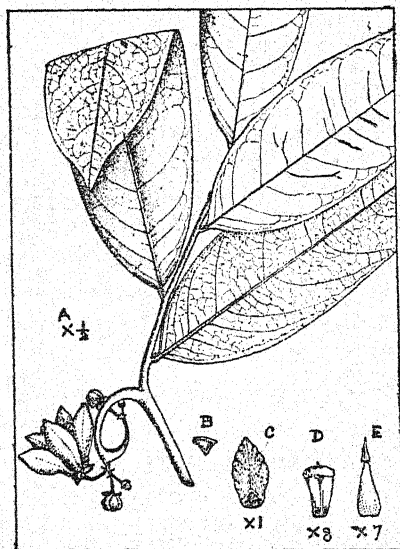
(Communicated by S. R. Bose)

Received for publication on December 14, 1939

WHILE working on a collection of plants from Assam and Burma at the Herbarium of the Royal Botanic Garden, Calcutta, the author came across a few plants which have proved new to science. Two of these are described below:—

### 1. *Artabotrys Cubittii* Chatterjee Sp. Nov.—

Habitu ab *A. multiflorus* C. E. C. Fischer (*Kew Bull.*, 1937, 436); sed ramis non insignis traversum, foliis majoribus, elliptico



*Artabotrys Cubittii* Chatterjee Sp. Nov. Fig. reduced half Nat.

A—Twig with inflorescence  $\times \frac{1}{2}$ . B—Sepal  $\times 1$ . C—Petal  $\times 1$ .  
D—Stamen  $\times 8$ . E—Carpel  $\times 7$ .



D. CHATTERJEE

lanceolatis, margini plano, sepala longa, connectivo staminum appendiculata, formato ovario differt.

A large climber forming sometimes a large bush of 2 to 2.5 m. high; branchlets rufous-tomentose when young, glabrous afterwards. *Leaves* shortly petioled, elliptic-lanceolate, acute or shortly acuminate, entire, narrowed at the base, upper surface glabrous or sparingly hispid when young, densely so on the veins, lower surface rufous hispid densely on the nerves, more or less glabrous when old; *lamina* 10-24 cm. long, 4-8 cm. broad, midrib prominent beneath, primary veins 9-12 pairs slightly raised below, anastomosing near the margin; *petiole* 3-7 mm. long, stout, slightly grooved, *Peduncles* somewhat flat, sharply curved twice,—first at the base, again a little about the middle. 1.5-2.5 cm. long, adpressedly rufous pubescent, bearing two 1-4 flowered fascicles, one at the apex and one at the second curvature. *Bracts* minute, lanceolate, pubescent; *pedicels* 8-15 cm. long, rufous hispid. *Sepals* deltoid, long-acuminate, 7-10 mm. long, rufous hispid on both sides. *Petals* 6, subequal, lanceolate, or ovate-lanceolate, obtuse, 18-25 mm. long, 7-10 mm. wide, concave at the base, the inner more so, conniving over and covering the stamens and carpels; thinly tomentose on both sides, densely on the base outside, glabrous on the concave part within, with a ridge of dense grey hairs above the concavity. *Stamens*, sessile, oblong-cuneate, 2 mm. long, grooved at the back, connective broad, flattened apiculate. *Carpels* 12, ovary pyramidal 4 mm. long, style constricted at the base and then abruptly dilated so that it appears to be articulated on the ovary. *Fruits* 1-3, shortly stalked, ovoid, narrowed at both ends, often obliquely compressed and shortly beaked. *Seeds* 1-2 in each fruit, plano-convex, elliptic, truncate at the base, 15-17 mm. long, 11-13 mm. broad; testa stony.

*Burma*.—Bhamo, alt. 163 m., 22-5-1910. *C. E. S. Cubitt* No. 618.

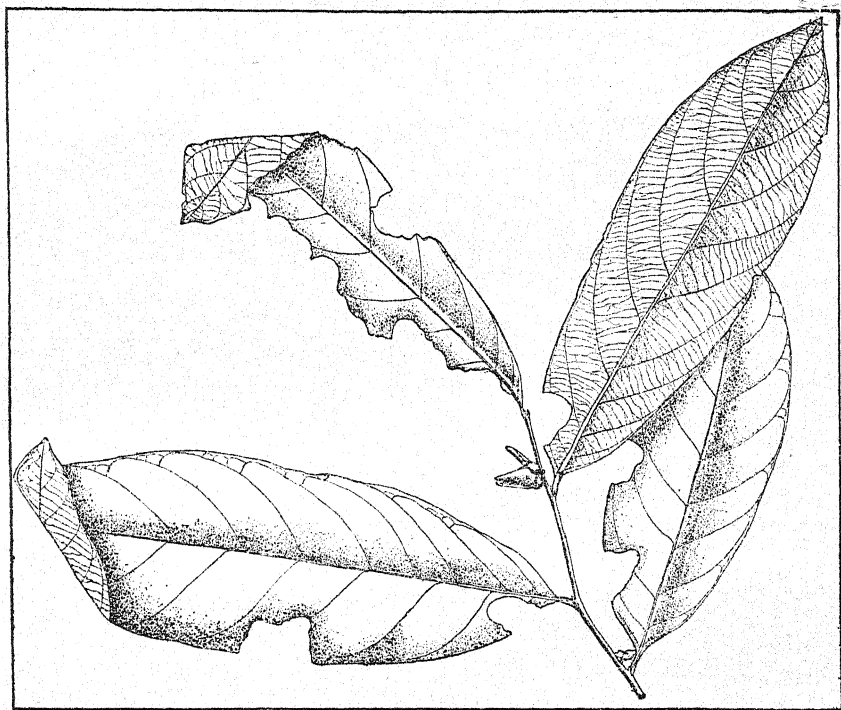
*Type in Herb. Cal.*, lower Chindwin, Kanni, alt. 130 m.; 7th Aug. 1908, *Lace* 4166; Ruby mines, Shanpanego left bank of Irrawaddy 5th July 1911, *Lace* Nos. 5314 and 5315; Myitkyina Indawgyi Reserve path from Nammun to Fepu, alt. 250 m.; 9th May 1919, *C. G. Rogers* No. 834.

*Assam*.—Manipur, Sittang Road, on the hills, 1890, *J. C. Prazer* No. 205.

2. *Oxymitra Biswasiana* Chatterjee Sp. Nov.—

*O. fornicata* Hk. f. & T. similis, sed foliis glaberimis, floribus minoribus, petalis subaequalibus, carpelibus minoribus differt.

Woody climber; stem and branches terete, black, lenticellate. *Leaves* simple alternate, shortly petioled, lanceolate or elliptic-lanceolate, acute, base tapering rarely, rounded, entire, glabrous on both surfaces, glaucous beneath; 8 to 10 pairs of nerves from the midrib, all conspicuously raised below; 7.5 to 16 cm. long, 2.5 to 6 cm. broad. *Petiole* 4 to 6 mm. long. *Flowers* extra axillary,



*Oxymitra Biswasiana* Chatterjee Sp. Nov. (Flowers small and subsessile)  
 $\times \frac{1}{2}$  Nat.

solitary, subsessile pyramidal. *Bract* solitary, ovate-lanceolate, situated just at the base of the calyx. *Sepals* 3, broadly deltoid, densely glandular, veined in the middle. Glabrous inside minutely pubescent outwards. 4.5 mm. broad, 4 mm. long. *Petals* 6, biseriate, alternate, ovate-lanceolate, entire, acute, minutely pubescent on both sides, outer petals somewhat flat, outer petals 12 mm. long, inner petals slightly shorter than the outer, 9 mm. long. *Stamens* numerous, linear, cuneate, truncate anther cells sublateral, top of connective large convex. *Torus* convex. *Carpels* 8 to 10—free, narrowly elliptic, densely pubescent all over, small, 1.5 mm. long, style highly compressed, conspicuously hairy at the top. *Ripe carpels* 5 to 6, ovoid, covered with rusty coating; 11–12 mm. long, 8–9 mm. broad, main stalk 5 mm., sharply bent secondary stalk 4 mm. long. Seed solitary ovoid, reddish brown shining with a median longitudinal depression.

Burma.—Tavoy, Heinze Chaung Camp. 550 m., *P. T. Russell*, No. 2095, *Type and Co-type in Herb. Calc.*, and 1814 (4 sheets).

Malacca.—Maingay 3397 (Kew distribution No. 61 Malaya).

*D. CHATTERJEE*

**THE**

The author takes the opportunity of dedicating the species in honour of Dr. K. Biswas, D.Sc., F.R.S.E., Superintendent, Royal Botanic Garden, Sibpur, Calcutta, whose valuable contributions to Indian Botany are numerous and under whose incentive the examination of some unidentified Burmese plants was taken up by the writer.

## DEVELOPMENT OF EMBRYO-SAC AND ENDOSPERM-HAUSTORIA IN SOME MEMBERS OF SCROPHULARINEÆ

*V. Ilysanthes hyssopioides* Benth., *Bonnaya tenuifolia*  
Spreng

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Received for publication on April 18, 1940

OBSERVATIONS on the present genera were communicated to the Indian Science Congress during its sessions of 1929 and 1931. A brief reference to them was also made by the author (1937) in his first paper of the series. Subsequently many other members of the Scrophularineæ have been studied, and accounts of their developmental peculiarities in the endosperm, haustoria etc., have been published by the author from time to time (1937, 1939*a*, 1939*b*, 1939*c*, and 1940*a*). In the last paper of the series an attempt has been made to classify these plants into the various types according to the method of formation of the endosperm haustoria. The two present genera can be brought under the *Pro-limosella*-type (GLIŠIĆ, 1936-37) as the following account shows.

### MATERIALS AND METHODS

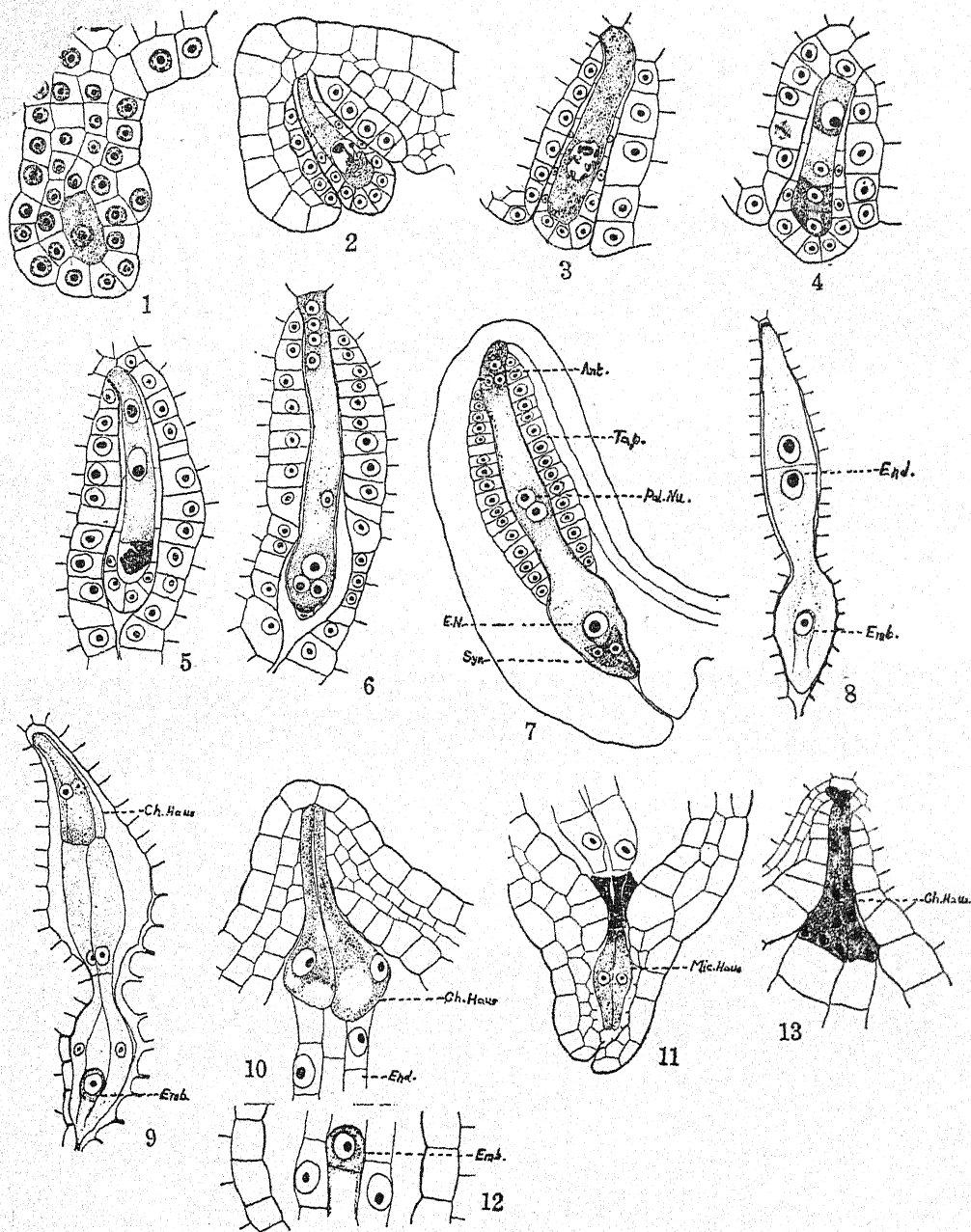
The material was collected from the marshes round about Bangalore and fixed in chromo-acetic acid solution with cmic acid. The sections were cut between eight and twelve microns and stained in Heidenhain's iron alum hæmatoxylin.

OVULE.—The ovary consists of a thick placenta bearing an indefinite number of anatropous ovules. The ovule is composed of a reduced nucellus and a single thick integument. The innermost layer of the integument develops into the tapetum which forms a lining layer to the lower non-dilated part of the embryo-sac. The cells of the placenta and the integument are filled with oil globules.

#### *Ilysanthes hyssopioides* Benth.

EMBRYO-SAC.—The large hypodermal archesporial cell, surrounded by a single layer of nucellar cells, functions directly as the megaspore-mother cell. The integument makes its appearance at this stage in the form of a ring at the base of the nucellus (Fig. 1), and as it develops further the ovule assumes an anatropous position. The megaspore mother cell elongates conspicuously, becoming broader towards the micropylar end and narrower towards the chalazal (Fig. 2). Fig. 3 represents a meiotic stage with the bivalent chromosomes; their

C. V. KRISHNA IYENGAR



FIGS. 1-13. *Ilysanthes hyssopioides* Benth.

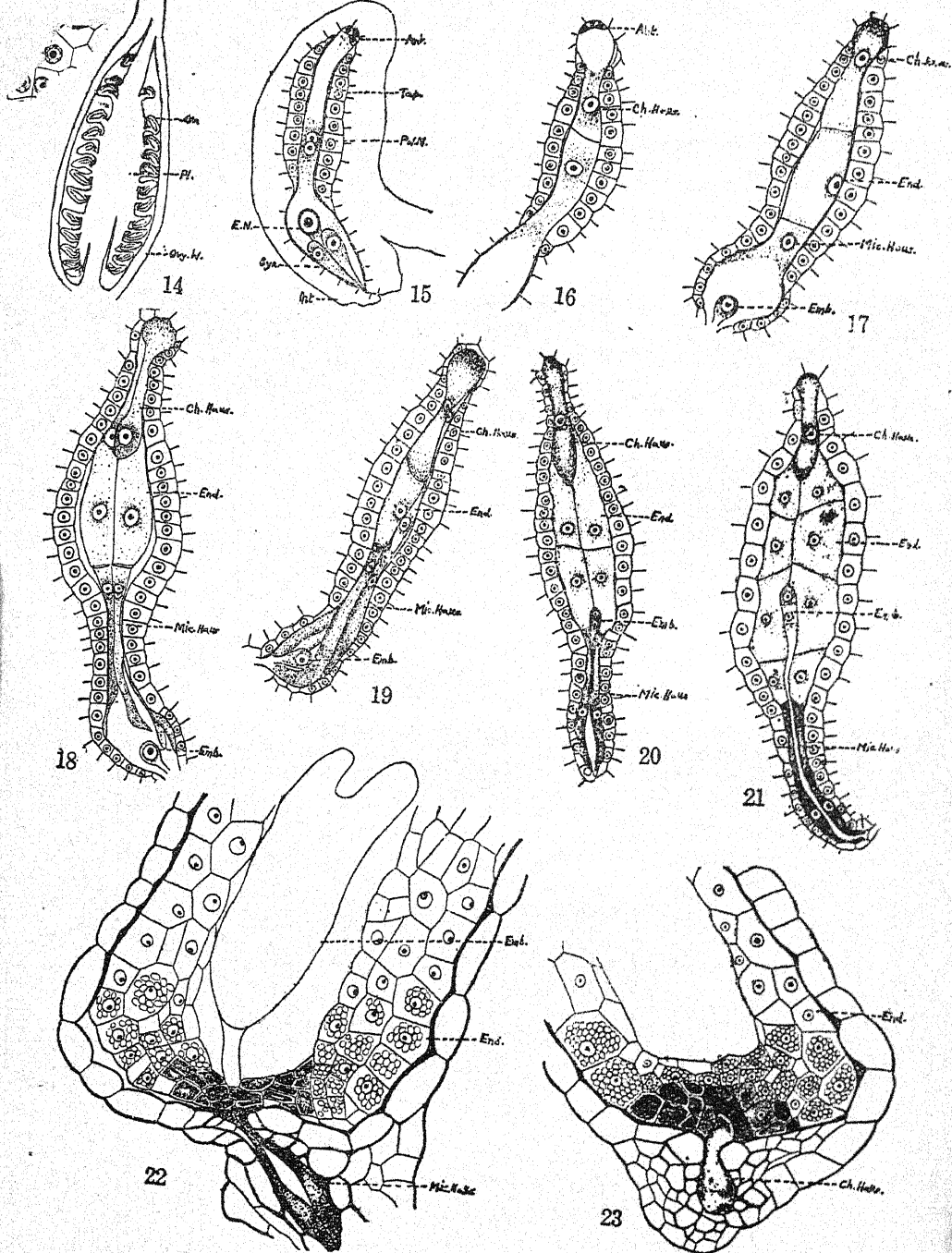
Figs. 1-4. Stages in embryo-sac development ( $\times 530$ ). Fig. 5. Stage in embryo-sac development ( $\times 233.5$ ). Fig. 6. Stage in embryo-sac development ( $\times 233.5$ ). Fig. 7. Embryo-sac ( $\times 233.5$ ). Figs. 8-13. Development of endosperm and haustorium ( $\times 140$ ). Ant. Antipodal cell; Ch. Haus. Chalazal haustorium; Emb. Embryo; E.N. Egg nucleus; End. Endosperm; End. N. Endosperm nucleus; Mic. Haus. Micropylar haustorium; Ov. Ovary; Ovy. wall. Ovary wall; Pl. Placenta; Pol. Nu. Polar nuclei; Syn. Synergid; Tap. Tapetum.

exact number could not be decided as the number of ovules showing this stage was very small in my material. The first division of the nucleus is followed by a transversely placed wall resulting in two dyad cells. Usually the second division takes place simultaneously in both the cells, resulting in a linear tetrad of megaspores. At times, however, one of the dyads divides earlier than the other. The outer three megaspores invariably degenerate and disorganise, while the chalazal enlarges and develops into the embryo-sac. Further nuclear divisions follow in quick succession resulting in a normal eight-nucleate embryo-sac. The two polar nuclei, now migrate towards the middle of the sac. The egg apparatus and antipodal cells are organised in a normal manner surrounded by dense cytoplasm. From the tetrad stage onwards the nucellar jacket steadily degenerates; even the vestiges of its cells are not seen when the embryo-sac is mature. The integumentary tapetum, already mentioned before, forms the lining layer of the narrower chalazal portion of the embryo-sac.

**FERTILIZATION.**—Although many flowers were collected and cut to study fertilization, the required stages could not be found in my material. The two polar nuclei fuse to form the secondary nucleus shortly before the entrance of the pollen tube. Immediately after fertilization the synergids and antipodals begin to degenerate. Even during the early stages in endosperm formation the disorganisation of these cells is complete.

**EMBRYO AND ENDOSPERM.**—The fertilized egg develops into a long tubular structure which squeezes itself between the micropylar haustorial cells and penetrates for some distance through the endosperm tiers. The further development of the embryo is quite normal and is similar to that of the other members of the family already studied by me.

The first division of the primary endosperm nucleus is succeeded by a transverse wall separating the chalazal chamber from the micropylar. The next division, which is also transverse, takes place in the micropylar chamber, and thus a row of three cells is organised. The middle one gives rise to the endosperm while the other two cells are the haustorial initials. The third division, which is longitudinal, takes place in all the cells. The fourth is also longitudinal, but this is confined to the micropylar and the middle cells. As a result of these four divisions ten cells are formed, namely, two chalazal, four middle and four micropylar ones, reminding one of the corresponding stages in *Alonsoa* and *Sopubia* (Author, 1937). After a series of longitudinal and transverse divisions the middle tier develops into the body of endosperm while the two terminal tiers form the micropylar and chalazal haustorial cells. During the development of the endosperm the embryo-sac enlarges significantly, adequate space being thus available for the enlarging endosperm tissue. The upper and lower ends of this tissue are composed of darkly staining smaller cells with rich protoplasmic contents but poor in starch. In all probability these cells assist in the translocation of food material from the haustoria to the more deeply placed endosperm tissue—a feature common to many members of Scrophularineæ. Starch grains are abundantly deposited in the endosperm cells.



FIGS. 14-23. *Bonneya tenuifolia* Spreng.  
 Fig. 14. L. S. of the ovary ( $\times 23.5$ ). Fig. 15. Embryo-sac ( $\times 140$ ).  
 Figs. 16-18. Development of embryo, endosperm and haustorium ( $\times 233.5$ ).  
 Figs. 19-23. Development of embryo, endosperm and haustorium ( $\times 140$ ).  
 Ant. Antipodal cell; Ch. Haus. Chalazal haustorium; Emb. Embryo;  
 E.N. Egg nucleus; End. Endosperm; End. N. Endosperm nucleus; Mic.  
 Haus. Micropylar haustorium; Ov. Ovary; Ov. wall. Ovary wall;  
 Pl. Placenta; Pol. Nu. Polar nuclei; Syn. Synergid; Tap. Tapetum.



ENDOSPERM HAUSTORIA.—The tier of cells towards the micropyle develops into the four uni-nucleate, unbranched micropylar haustoria. These are spindle-shaped bodies aggressive in nature, as seen by the disorganised cells in the neighbourhood. These show rich contents and hypertrophied nuclei and persist long after the chalazal haustoria show degeneration.

The two cells towards the chalaza form the chalazal haustoria. Their upper ends are large and bulbous and the lower are drawn out into simple long and unbranched tubular structures ending just near the vascular traces of the integument (Fig. 10). The rich protoplasm is somewhat vacuolated in the upper end, and the hypertrophied nuclei are very conspicuous. The vertical separating membrane between the haustoria is very thin. Even during early stages of endosperm formation the chalazal haustoria show signs of senility. Their nuclei disintegrate resulting in the coenocytic appearance of the haustoria, which at this stage appear partly reduced in size.

*Bonnaya tenuifolia* Spreng.

EMBRYO-SAC.—The embryo-sac is exactly similar to that of *Ilysanthes*. The dilated micropylar end, the narrow chalazal end lined by the integumentary tapetum and containing three small antipodals, and the fusion of the polar nuclei before fertilization, are common to both the plants.

EMBRYO AND ENDOSPERM.—The stages in the embryo formation are also similar to those of *Ilysanthes*.

As before, a row of 3 cells is formed after the division of the primary endosperm nucleus. The middle cell gives rise to the body of the endosperm while the terminal cells give rise to the haustoria. As before the two ends of the endosperm tissue are composed of smaller cells probably meant to act as translocatory cells.

HAUSTORIA.—The first four divisions of the primary endosperm nucleus result in the organisation of two chalazal, four micropylar and four central cells. The micropylar haustoria are simple, unbranched, uni-nucleate and persistent. The chalazal haustoria are simpler than in *Ilysanthes*, and do not show the differentiation into a swollen bulbous region and a long drawn out lower region. Older haustoria show the disintegration of the two nuclei and the dissolution of the separating membrane resulting in a single large chamber in this region.

In the mature seed the entire tissue of the integument including the tapetum is found to be used up and absorbed, so that the endosperm cells come in direct contact with the epidermis which shows conspicuous thickening of its cell walls.

#### CONCLUSION

The position of the two closely allied genera *Ilysanthes* and *Bonnaya* in relation to the other members of the family may be summed up as follows:—



**ARCHESPORIUM.**—The presence of a single archesporial cell which develops into the megaspore mother cell is common to all the members. Tetrad formation from a single mother cell and the subsequent development of the chalazal megaspore take place without any notable variation. Occasional deviations from this are seen in many parasitic members of the Rhinanthæ (SCHMID, 1906), where the occurrence of more than one archesporial cell and the consequent formation of several tetrads happens to be a noteworthy feature.

**EMBRYO-SAC.**—The development of the sac is entirely normal in the whole family. The antipodal cells are generally small and evanescent. *Stemodia* (Author, 1939b) is an exception, since here the chalazal end is significantly large and shows three large antipodals loosely placed in this region. In *Alectorolophus* (SCHMID, 1906) only two antipodals are present, one of which is bi-nucleate owing to the absence of a separating wall. In *Melampyrum* (SCHMID, 1906) antipodals are only rarely found in the mature embryo-sac, although the exact reason for this is not known. The frequent occurrence of a narrow chalazal end, and the constant presence of small antipodals associated with this, lead one to infer that want of adequate space is responsible for the reduced size of the antipodals.

The occurrence of starch grains in the mature embryo-sac is noticed in several members, namely, *Alonsoa*, *Stemodia*, *Isoplexis*, *Celsia*, *Limnophila* and *Vandellia*. The author is of opinion that the delay in the organisation and functioning of the haustoria may be responsible for this. In the recent compilation of DAHLGREN (1939), starch grains are mentioned to occur in only 54 families. Unfortunately, he mentions only a single member of the Scrophularineæ, and this is from the author's first paper in the series (Author, 1937).

**HYPOSTASE.**—The formation of a nutritive tissue at the chalaza is reported in *Verbascum*, *Celsia*, *Isoplexis* and many other plants of the family. This is composed of radiating cells with rich protoplasmic contents. This condition is associated with a reduced chalazal haustorium in these plants. Whether it is the presence of this compact tissue which impedes the growth of the haustoria, or the aid rendered by this in the absorption and transportation of the nutritive material which makes extra development of the haustorium unnecessary or superfluous, are questions that cannot be definitely answered.

**FERTILIZATION.**—The two polar nuclei generally fuse together to form the secondary nucleus which shows a tendency as in *Alectorolophus* and others to migrate to the neighbourhood of the egg either before or after fertilization. There are also instances like *Scrophularia*, *Veronica*, etc. (SCHMID, 1906) where the polar nuclei are free till fertilization is over. In most of the types studied so far the antipodal cells and synergids degenerate and disorganise immediately after fertilization. In *Stemodia* and *Vandellia* the antipodals persist during the early stages of endosperm formation

while in *Gratiola* (GLIŠIĆ, 1933) these are reported to be present even longer during older stages of endosperm and embryo development.

**INTEGUMENTARY TAPETUM.**—During the development of the embryo-sac the nucellar jacket begins to degenerate till at last the sac lies in direct contact with the integument. There are, however, some exceptions. In *Isoplexis* for instance (Author, 1939a), the occurrence of this jacket with starch grains in its cells has been noted even when the embryo-sac is mature.

The development of the integumentary tapetum and its nutritional value has been described by several investigators. The presence of rich protoplasm, starch grains or fat globules characterises the cells of this layer. It often lines the non-dilated chalazal part of the mature embryo-sac although the entire sac is surrounded during the earlier stages. *Stemodia* (Author, 1939b) is exceptional, for here the chalazal end is dilated and devoid of the tapetum while it is the micropylar part which shows the sheath. In *Alectorolophus* sp. (SCHMID, 1906) the entire sac possesses a sheath while *Pedicularis* sp. (SCHMID, 1906) shows varying grades of sheath formation. In several members the later stages of tapetum are particularly interesting. In *Verbascum* (SCHMID, 1906), *Celsia* (Author, 1939a) and *Vandellia* sp. the cells are of significantly different sizes. Smaller and larger cells alternate regularly in *Verbascum*, but in *Celsia* and *Vandellia* this regularity is wanting. Generally, in all the three cases, the larger cells occur in the middle portions of the sheath. In *Centranthera* (Author, 1939c), however, a few cells at the chalazal end of the sheath enlarge conspicuously. It is often noticed that the unusual development of the tapetal cells takes place at a time when the haustoria are unable to meet the demands of the growing embryo and endosperm, either on account of their reduced size or senility or nonfunctional nature. A digestive, absorptive and storage function has been ascribed to the tapetum in these members, the peculiar thickening of the tapetal cell walls in this region and the enlargement of the cells lending further support to this view. Since the tissue between the tapetum and epidermis is digested and absorbed, and since the epidermis does not show any thickening of its cell walls in several members, the protective rôle has also to be performed by the tapetum. In *Limnophila*, *Stemodia* (Author, 1939b) and *Bonnaya*, on the other hand, it is the epidermis which assumes the mechanical rôle.

**ENDOSPERM AND HAUSTORIA.**—The sequence of early divisions of the primary endosperm nucleus during the development of endosperm and haustoria has engaged the attention of many investigators. In all the types studied so far the first division of the nucleus is followed by a transversely placed wall separating the embryo-sac into a chalazal and a micropylar chamber. The succession of other divisions varies in different members. The following table and diagrams summarise the conditions found in all the plants studied by me so far. From this it is seen that the second division is transverse in *Alonsoa*, *Ilysanthes*, *Bonnaya*, *Sopubia*, *Vandellia* sp.

(Author, 1937, 1939c, and 1940a) and *Herpestis* (SRINATH, 1934), and this separates the "Endosperm mother cell" from the micropylar chamber at a very early stage. Thus, we find three chambers, chalazal, the middle and the micropylar after the first two divisions. *Pedicularis* (SCHMID, 1906) and to a certain extent *Lathræa* show the same sequence, but in the latter the second division is sometimes longitudinal instead of transverse. *Isoplexis*, *Celsia*, *Verbascum* (SCHMID, 1906) and *Scrophularia* (SCHERTZ, 1919) belong to the *Verbascum*-type showing two longitudinal divisions succeeding the first transverse one. *Limnophila* and *Stemodia* conform to the *Limosella*-type (GLIŠIĆ, 1936-37). In the *Verbascum*- and *Limosella*-types the differentiation of the endosperm cells from the micropylar haustorial cells is delayed. Since *Lathræa* also occasionally exhibits a similar feature one is led to suspect that the nutritional factor might have a part in this peculiarity. I am inclined to believe that a few nutritional experiments conducted on some members may yield interesting results in this line.

The number and formation of the haustoria also varies in the several types. The earliest to be organised is the chalazal haustorium while the micropylar haustorium is differentiated later on. The most common and basic number of haustorial cells is four micropylar and two chalazal ones. Even if this number be modified, the number of nuclei remains the same. Thus, in the micropylar haustorium it is possible to find all grades from four uni-nucleate cells to one tetra-nucleate body. A tetra-nucleate chalazal haustorium is, however, not seen,\* although four uni-nucleate cells are present in the *Verbascum*-type. The multi-nucleate condition is the result of either nuclear division or incomplete wall-formation or a dissolution of the separating membrane between the haustorial cells. The bi-nucleate stage of the chalazal haustorium and the bi- or tetra-nucleate stage of the micropylar haustorium are instances of this kind. An interesting exception is seen in *Gratiola* (GLIŠIĆ, 1933), in which the chalazal haustorium, instead of remaining a single uni-nucleate cell, occasionally forms two uni-nucleate cells or a single bi-nucleate body. A similar condition is seen in *Vandellia* in which a single uni-nucleate chalazal haustorium may be present either from the commencement, or is formed at a later date by the degeneration of one of the nuclei of a bi-nucleate cell. The exact reason for the degeneration of the nuclei cannot be stated at present.

The sequence of the haustoria is another noteworthy feature. The chalazal haustorium is organised first and the micropylar is a later arrival; to correspond with this the senility of the chalazal haustorium also sets in earlier. The micropylar haustorium is often more elaborate in structure than the chalazal one (although the latter is considerably larger in several members) and assumes various shapes. This is highly branched in *Alonsoa*, bulbous in

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\* The author has subsequently observed a tetra-nucleate chalazal haustorium along with the secondary haustorial filaments in *Angelonia grandiflora*. A paper on this plant is ready for publication.

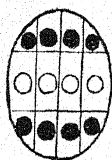
Genus	Author	Year	Sequence of Divisions				Mic. Haus. Cells		Ch. Haus. Cells		Early stage	Late stage	
			1st	2nd	3rd	4th	Early stage	Late stage	Early stage	Late stage			
<i>Isoptericis</i>	C.V.K. Iyengar	1939 <i>a</i>	Tr.	Long	Long	Tr.	4	4	4	4			
<i>Celsia</i>	"	"	"	"	"	"	4	4	4	4			
<i>Alonsoa</i>	"	1937	"	Tr.	"	Long	4	4	4	2	1		
<i>Ilysanthes</i>	"	"	"	"	"	"	4	4	4	2	1		
<i>Bonnaya</i>	"	"	"	"	"	"	4	4	4	2	1		
<i>Sopubia</i>	"	1937	"	"	"	"	4	1	4	2	1		
<i>Vandellia hirsuta</i>	"	1940 <i>a</i>	"	"	"	"	4	1	4	2	1		
<i>V. scabra</i>	"	"	"	"	"	"	4	1	4	2	1		
<i>Linnophila</i>	"	1939 <i>b</i>	"	Long	"	Tr.	4	1	4	1	1		
<i>Stenodia</i>	"	"	"	"	"	"	4	1	4	1	1		

*Isoplexis*, club-shaped in *Bonnaya* and *Ilysanthes*, tubular, filiform and prong-like in *Melampyrum* (SCHMID, 1906) and molar-like in *Paulownia* (MILLSAPS, 1936). Often one kind develops at the cost of the other. In the author's words (1937) "the kind of haustorium present, its shape, size and structure, and the duration of its activity depend upon the degree of demands made by the developing embryo and endosperm on the stored up material, their period of dependance, the available nutrition in the neighbourhood and the quality of nutrition". There are aggressive as well as non-aggressive haustoria present. In all the parasitic *Rhinanthæ* both the haustoria are often aggressive. In the *Verbascum*-type these are non-aggressive, while in *Gratiola* they are reported to be almost non-functional.

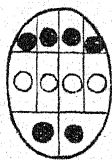
The older haustoria often show hypertrophied nuclei. Although the nuclear size is inversely proportional to the number of haustorial cells it is found that the sizes of the haustorium and nucleus cannot be correlated. The hypertrophy of a nucleus is understood to be the result of its response to the nutritional stream; but it is not clear to me why one of the nuclei in the chalazal haustorium of *Vandellia* should degenerate. A similar situation is met with in *Mentha* (RUTTLE, 1931) in which one of the nuclei of the bi-nucleate chalazal haustorium happens to be smaller than the other.

The presence of cellulose rods or granules in the older haustoria is a common feature of many members. It reaches its highest development in the 'lateral haustoria' of *Pedicularis* (SCHMID, 1906), forming a conspicuous net-like structure. SCHMID attributes a mechanical function to these structures since their advent coincides with the time of the haustorial senility. But in *Vandellia hirsuta* (Author, 1940a) while the chalazal haustorium begins to degenerate most of these rods also disappear, leading one to infer that they have a probable nutritional rôle.

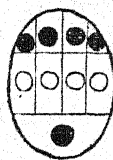
The evolutionary tendency of the haustoria in the *Scrophularinæ* is an important feature. A few diagrams have been presented below to show the condition in the different forms. The *Verbascum*-type seems to be the most primitive including *Isoplexis*, *Verbascum*, *Celsia*, and others, showing four haustorial cells of each kind. The



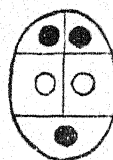
*Verbascum*-  
type



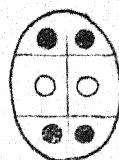
*Pro-Limosella*  
(*Alonsoa*)-type



*Limosella*-  
type



*Gratiola*-  
type



*Paulownia*-  
type

next in the series is the *Pro-limosella*-type including *Alonsoa*, *Ilysanthes*, *Bonnaya*, *Vandellia* and others all of which show four micropylar haustorial cells and two chalazal cells the later fusing to form a single bi-nucleate body at a later stage. *Vandellia* is

exactly like *Alonsoa* in having two uni-nucleate chalazal cells, which later form a single bi-nucleate cell by the disappearance of the separating wall, reminding one of *Linaria* (SCHMID, 1906) and shows a uni-nucleate condition later on as in *Limosella* (SVENSSON, 1926), *Stemodia* and *Limnophila* (Author, 1939b). The next stage is seen in the *Gratiola*-form where the chalazal haustorium is a uni-nucleate cell and the micropylar is composed of two uni-nucleate cells. Occasionally a bi-nucleate cell or two uni-nucleate cells are reported in the chalazal haustorium of *Gratiola*, which feature might throw some light on the ancestry of this member. *Paulownia* (MILLSAPS, 1936) stands as a unique type with the incomplete cell division in both the haustoria which happen to be bi-nucleate as in the figure. While this form occupies a higher place than *Gratiola* (if only the number of cells is taken into account) it ought to be less advanced judging from the nuclear number. Thus the tendency seems to be to reduce the number of haustoria and their nuclei. Although the occurrence of a single uni-nucleate chalazal cell is a common feature in many cases, the micropylar haustorium is composed of either two uni-nucleate cells or a single bi-nucleate body even in the most advanced members of the family, pointing out thereby that the micropylar haustorium is a more conservative structure in this family as a whole. While there is not a single *Scrophulariaceous* member with one uni-nucleate micropylar haustorium, there are several instances of this kind in the Bignoniaceæ (MAURITZON, 1937).

Interesting deviations in the function of the haustorial cell are reported in several members. The addition to the endosperm tissue by the activity of the primarily haustorial cell as in *Incarvillea compacta* (MAURITZON, 1937) and *Utricularia* (KAUSIK, 1938) and the formation of conducting cells from the chalazal and the micropylar haustorial cells as in *Gratiola* are some of the peculiar functions of the haustoria.

#### SUMMARY

1. A reduced nucellus and massive integument are present in both the plants. The formation of a tetrad of megaspores and development of the embryo-sac take place normally. The nucellar epidermis disorganises at a very early stage and the integumentary tapetum lines the narrow chalazal end of the embryo-sac.

2. The primary endosperm nucleus gives rise to three chambers by two transverse divisions accompanied by wall formation. Two longitudinal divisions follow, resulting in the formation of the micropylar, chalazal and middle tiers of cells. The middle tier develops into the body of endosperm.

3. The chalazal cell divides to form two unbranched uni-nucleate chalazal haustoria. In *Ilysanthes* these are broad and bulbous towards the endosperm and tubular towards the chalazal; in *Bonnaya* these are simpler in structure.

4. The micropylar tier develops into four unbranched uni-nucleate haustorial cells which are spindle-shaped or club-shaped. These are aggressive in *Ilysanthes* and non-aggressive in *Bonnaya*.



5. The degeneration of the chalazal haustorium sets in earlier in both the forms, and the thin separating membrane shows a tendency to disorganise, the haustorium in the meanwhile presenting a multi-nucleate appearance by the fragmentation of its nucleus as in *Ilysanthes*.

6. The endospermal cells adjacent to the haustoria are smaller and more richly protoplasmic. In all probability their function is to assist in the translocation of the food material from the haustoria to the more deeply placed endosperm tissue.

7. The embryo in both the forms is typically dicotyledonous.

Grateful acknowledgement is made to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M. (Chicago), Professor of Botany, Central College, Bangalore, who was kind enough to suggest this problem and give all facilities for its investigation, and to Dr. P. Maheshwari, D.Sc., F.N.I., of the University of Dacca, for his suggestions and helpful criticism while writing this paper.

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## STUDIES ON THE PHOTOCHEMICAL ACTION IN PLANTS

### I.—The respiration of entire *Pistia* plants in light

BY SHRI RANJAN

Received for publication on July 13, 1930

#### INTRODUCTION

SOME of the earliest works in connexion with the effect of light on the respiration in plants comes from Russia. It was Borodin<sup>3</sup> who discovered an indirect relation between light and respiration. He found that the respiratory activities of leafy twigs, when kept in darkness, gradually decreases, while the activity augments when they are again illuminated. He suggests this to be due to the decrease and increase of carbohydrates in darkness and light respectively.

Bonnier and Mangin<sup>2</sup> however showed that light has a direct effect also on the respiratory mechanism. If plants are placed alternately in light and darkness a retarding influence of light is noticed. This has no relation to carbon assimilation for the effect is noticed in plants without chlorophyll also.

Maximov<sup>6</sup> noticed that the effect of light on *Aspergillus niger* varies with the age of the culture as also with the nature of nutrient medium.

Recently Middleton<sup>7</sup> and Whimster<sup>13</sup> noted a very slight effect of light which they ascribe to ionising action of light.

Carreau also noted the liberation of CO<sub>2</sub> from tissues in light. Cerighelli,<sup>4</sup> however, interpreting his results, believed that this is due to the liberation of CO<sub>2</sub> from non-green tissues when the temperature is high. When the temperature however is below 30° C there is no CO<sub>2</sub> emission which is due to the assimilation exceeding the respiration rate.

Spoehr<sup>11</sup> divided the light reaction into two classes:—the direct and the indirect. In the first light directly acts bringing about physico-chemical changes of certain physiologically important substances within the organism *e.g.*, reduction of pH, etc.

Meyer and Deleano found that at constant temperature and in dark CO<sub>2</sub> production is decidedly higher during day than at night. Mortiz and Traube believe that it is the partially dissociated oxygen (-o-o-) which combined with the oxidisable substances.

Warburg<sup>12</sup> however found that light has some photochemical effect on respiration. Working on yeast he found that the respiration was arrested by the presence of CO. But if a mixture of CO

and yeast was exposed to light, respiration restarted. This he said was due to the dissociation in light of the CO which is bound to the Fe of the respiratory ferments.

The researches of Dhar<sup>5</sup> and his collaborators, *in vivo*, have shown that food materials like starch, sugar, proteins and fats in aqueous solutions or suspensions are oxidised to carbon dioxide and water by simply passing air at ordinary temperature in presence of light. In the absence of light they find no such oxidations. In the presence of inductors, however, like ferrous hydroxide or glutathione, the food materials are oxidised even in the absence of light, but in the presence of light these induced oxidations are greatly augmented. Dhar has also been able to show that Einstein's Law of photochemical equivalence has been found to be applicable to the photo-oxidation of glucose. Recently Abilous, Aloy and Valduque<sup>1</sup> have shown that lævulose and other sugars obtained by hydrolysis of carbohydrates readily decompose in sunlight with the formation of formaldehyde.

Regarding the influence of temperature on photochemical reactions, in ideal cases, in the absence of light, no chemical change should take place, and so the temperature coefficient should be unity. In such cases Einstein's Law of photochemical equivalence will hold good. But such ideal reactions are very rare. Generally the temperature coefficient of a reaction occurring in light is much greater than unity but it is smaller than that of the reaction in dark. Dhar finds, in the case of oxidation of potassium oxalate by iodine, that in darkness for a 10° C rise, it has a value of 7.2. While in diffuse light the temperature coefficient is 3.4. This is due to the previous activation of most molecules in light and consequently further activation is relatively less with rise of temperature.

Parija and Saran<sup>8</sup> working on the albino varieties of *Aralia* found that, by exposing these plants even to a short period of diffuse light, the respiration rate after the exposure got augmented.

While not aspiring to clarify fully the physico-chemical complexities involved in the light reaction an attempt is made in this paper to gauge the effect of light, at different temperatures on the respiration of plants. Work on this line is fraught with difficulties, for no sooner are the green tissues exposed to light than the reverse reaction, *i.e.*, assimilation commences, masking in some cases completely the respiration rate of the tissues.

#### METHODS AND PROCEDURE

For the determination of the rate of respiration, the method of estimating the amount of carbon dioxide given out by the leaves, in a current of air, was employed. For this purpose the well-known air current commutator devised by Blackman was used. The flow of air through the plant chamber was maintained by an aspirator at a rate of about 1000 c.c. per hour.

The plant chamber was a simple large mouthed bottle, fitted with a rubber cork, through which inlet and outlet tubes were

inserted. This was placed in a Hearson's cool incubator and the temperature regulated according to the needs of the experiment.

As a source of light 1000 w. Osram lamp was used and was placed at a distance of one foot from the respiration chamber.

A rectangular glass jar through which circulated a stream of cold water was interposed between the lamp and the respiration chamber which absorbed the radiant heat from the lamp.

#### THE PLANT MATERIAL

The material used was *Pistia* belonging to the family Araceæ. The size of the plant is ideal for experimental work with entire plants. It is free floating possessing a rosette of green leaves. For each experiment a full grown healthy *Pistia* was brought from the garden in a beaker containing water. The plant was well washed and then put in the plant chamber which contained about 4 c.c. of distilled water. After each experiment the plant was taken out and the roots removed. It was then dried at 95° C in an electric heater and its dry weight taken. As far as possible full precautions were taken to ensure uniformity regarding the selection of plant materials for the successive experiments.

#### EXPERIMENTAL RESULTS

##### *Respiration of Pistia Shoots :—*

*Experiment 1.*—The first experiment conducted on *Pistia* was at 35° C. The respiration which started at about 11.7 mg. CO<sub>2</sub> per gr. dry weight per 3 hours, fell off within 30 hours to a value little above 5 mg. CO<sub>2</sub> (Fig. 1).

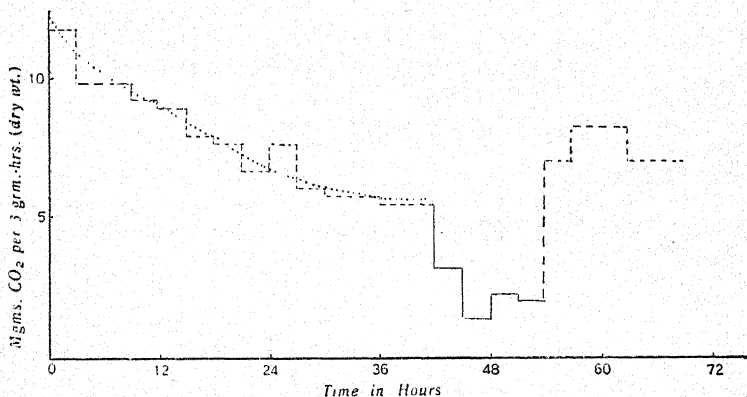


FIG. 1 (35° C)

- ..... Smoothed out curve
- Respiration in dark
- CO<sub>2</sub> given out in light

After a period of 42 hours in darkness, when light was supplied, the  $\text{CO}_2$  emission fell off due to photosynthesis in successive steps to a minimum value which was 1.3 mg.  $\text{CO}_2$  per gr. dry weight per 3 hours. However, after remaining at this low value for a brief period of 3 hours it again rose slightly. The change from light to darkness brought about a rapid rise in the  $\text{CO}_2$  output. The maximum  $\text{CO}_2$  emission at this stage being higher than the protoplasmic respiration just before exposure to light. However, after having attained this peak the respiration rate again fell off almost at once.

*Experiment 2.*—This experiment was carried out at  $40^\circ\text{C}$  and is shown graphically in Fig. 2. Here the respiration rate from the very beginning was less than the rate of respiration of the previous experiment.

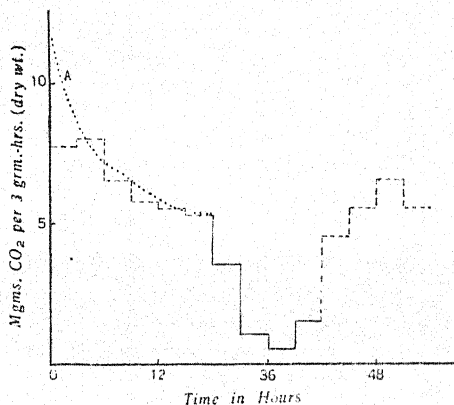


FIG. 2 ( $40^\circ\text{C}$ )

..... Smoothed out curve  
 ----- Respiration in dark  
 —————  $\text{CO}_2$  given out in light

The observed  $\text{CO}_2$  emission decreases, in light, more or less, in the same form as in the preceding experiment, *i.e.*, dropping to a minimum rate which is 0.5 mg.  $\text{CO}_2$  and then again rising slightly.

After light when the plant was put in darkness, the respiration rate steadily rose higher and higher reaching a maximum of 6.5 mg.  $\text{CO}_2$  between 6 and 9 hours. Thereafter it fell off.

A point of similarity between the previous experiment and this one is that in both cases the protoplasmic respiration comes down to nearly 5 mg.  $\text{CO}_2$ . The difference, however, between the two is in the after effect of light. In the preceding experiment the after-effect of light is very much more marked than in this.

*Experiment 3.*—For the following experiment below room temperature ice was put in the incubator and the temperature regulated at  $27^\circ\text{C}$ .

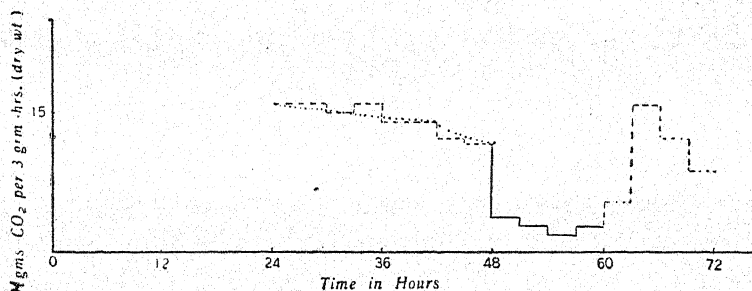


FIG. 3 (27° C)

..... Smoothed out curve  
 ----- Respiration in dark  
 ————— CO<sub>2</sub> given out in light

To avoid taking unnecessary readings of the floating part of respiration, the plant was put as usual in the plant chamber, but the air issuing from it passed through distilled water placed in the pettenkofer tubes. It was only after 24 hours that the respiration readings were taken. The respiration rate, at the protoplasmic level, is only 3.8 mg. CO<sub>2</sub>. This is markedly less than what it is at 35° C or 40° C. After a total period of 48 hours when the respiration rate had come down to a steady protoplasmic level, light was given. In light CO<sub>2</sub> emission drops, as usual in successive steps to a minimum of 0.6 mg. CO<sub>2</sub> thereafter it again rises slightly. The after-effect from light to darkness is well marked and shows a maximum at the end of 3 hours. Soon after the peak is touched the respiration rate commences to climb down.

*Experiment 4.*—The last experiment of this series was made at 20.5° C. The plant, as in the previous experiment, was first

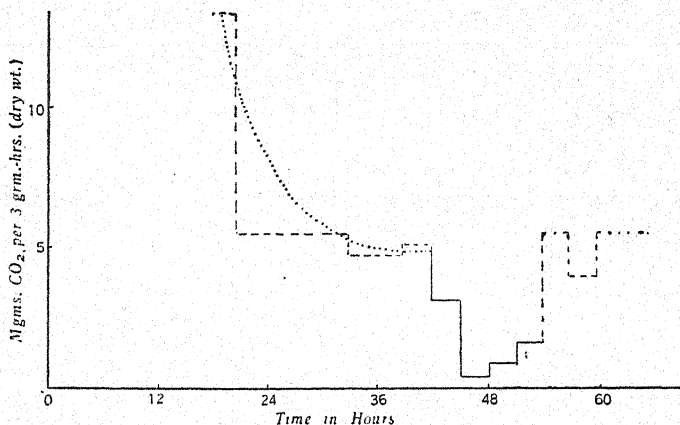


FIG. 4 (20.5° C)

..... Smoothed out curve  
 ----- Respiration in dark  
 ————— CO<sub>2</sub> given out in light

put in darkness for 18 hours at room temperature (nearly 32° C) but no readings were taken.

The respiration rate (Fig. 4) which showed high value at first, at 20.5° C, quickly climbed down to about 5 mg. CO<sub>2</sub>. After 24 hours at 20.5° C light was given. The CO<sub>2</sub> emission fell off as usual to a minimum of 0.4 mg. CO<sub>2</sub> and thereafter steadily increased. After 12 hours in light the respiration in darkness did not show much of an after-effect in this case. The respiration rate reached 5.5 mg. CO<sub>2</sub>, where it kept constant till the end of the experiment.

#### *Respiration of Pistia Roots :—*

*Experiment 5.*—In order to see whether the respiration of non-green tissues is as high as that found in green tissues the investigation of the respiration rates of *Pistia* roots was undertaken. As *Pistia* grows in ponds it was easy to find out the CO<sub>2</sub> output and O<sub>2</sub> intake of these roots without damage. The top green portion was removed from the lower portion with roots which was then placed in a beaker of water in the sun for about 2 hours. It was then well washed and placed in a respiration chamber. The estimations of dissolved CO<sub>2</sub> and O<sub>2</sub> were made by Winkler's method as improved by Pal.

The results are given in a tabulated form below.

TABLE I

Time	CO <sub>2</sub> output per hour mgms.	CO <sub>2</sub> per 3 hours mgms.	O <sub>2</sub> intake per hour mgms.	O <sub>2</sub> per 3 hours mgm.	$\frac{\text{CO}_2}{\text{O}_2}$ after conversion from weight to volume
9-30—10-30 A.M.	1.69	5.07	1.17	3.51	1.05
12-5 — 1-15 P.M.	1.69	5.07	1.3	3.9	0.94
3-0 — 4-0 P.M.	1.39	5.07	1.26	3.78	1.03

From the table one finds that the respiration is low being about 5 mg. CO<sub>2</sub> per gm. dry weight per 3 hours which is about the same as the protoplasmic respiration of green tissues at 35° C. Another significant feature is the total absence of the floating part of respiration, there being no evidence of a fall with time, even though the roots were previously exposed to sun light for two hours.

#### DISCUSSION OF THE RESULTS

Photochemical reactions according to Helmholtz can be divided into two main groups, *viz.*, (1) in which light accelerates a transformation which takes place in the dark and hence there is a



diminution of free energy, (2) in which light causes a reaction to take place and thus light supplies the necessary energy.

The reactions in the first case are *exoenergetic* reaction—such reactions are capable of giving out energy. In the second case they are *endoenergetic*, and here there is an increment of free energy. In plants the well-known case of endoenergetic reaction is carbon assimilation. We have to see whether the respiration of the tissues can in any way be a photochemical reaction of the exoenergetic type. For this purpose we have studied the problem from four different angles, *viz.*,

- (1) The floating part of the respiration.
- (2) The respiration of non-green but coloured part of plants in light.
- (3) The after-effect of light on respiration.
- (4) The effect of temperature and light upon respiration.

The first three of these have been dealt with else where by the author<sup>9</sup> when the idea of photochemical reaction for respiration was first conceived. Here we shall deal with these only succinctly in the light of work done subsequently to lead us on easily to the understanding of the temperature and light effect upon respiration.

### 1. The 'Floating' Part of Respiration

#### (a) The Photochemical Stage.—

Most plants, respiring in darkness, show a logarithmic type of fall in the respiration rates when brought from light to darkness. This unstable transitory phase is noticed only at the initial stages of respiration and is called the 'floating' part of respiration by Blackman. This is followed by the protoplasmic respiration rate of Blackman's which is the lowest plane of respiration reached in darkness.

That starvation is definitely not responsible for the immediate fall in the 'floating' part of the respiration curve, is shown by Ranjan<sup>9</sup> for *Mangifera* and *Eugenia* leaves. Naturally the leaves of these plants possess enough food material in reserve to maintain a high rate of respiration for weeks.

Physiological starvation, however, which can be brought about by high temperature due to the change in the starch-sugar balance, is well known. But our laboratory temperature of 35° C was, if anything, less than the temperature at noon in the open under a blazing sun. Thus physiological starvation too will not explain the fall in the respiration rate.

In the case of starchy leaves of Angiosperms it is generally noted, that though there is a slight fall of the monosaccharides during the 'floating' part of respiration, there is still enough sugar to maintain the respiration at a high rate.

Thus the fall in the respiration rate, which is generally always noticed no sooner than the leaves are brought from light to darkness, may be due to the direct effect of solar radiations on some stage of



plant respiration. That being so, we must then assume that along with the pure chemical or thermal change there is also a distinct photochemical stage in respiration.

(b) *The Respiration Rate of Roots :—*

The respiration rate of the *Pistia* roots confirm the belief that the protoplasmic respiration is nothing else but the respiration rate in darkness. From Table I, one finds that the respiration rate is about 5 mg.  $\text{CO}_2$  per gr. dry weight per 3 hours. This corresponds to the protoplasmic respiration of the *Pistia* shoots at  $35^\circ \text{C}$ . One notices also in the case of the respiration of the roots that there is no fall in the successive respiration readings. Now, as protoplasmic respiration, in the case of green leaves, is the final and the real respiration rate in darkness, there can be no further fall. In the case of the roots, as they possess no chlorophyll, there can be no photo-oxidation and hence the 'floating' part of respiration is totally absent.

2. *The Respiration of the Non-Green Leaves in Light*

(a) *The Photochemical Oxidation in the Yellow Leaves of Croton :—*

The study of the photochemical effect upon respiration of colourless excised leaves of *Croton* (Ranjan<sup>9</sup>) further supports the view of the photochemical activation of the reacting molecules in respiration. Some varieties of *Croton* leaves are more or less colourless having just a tint of yellow. These were selected for experimentation. Their respiration rate showed, at the floating part, the same type of fall as has been described in the preceding pages. When they were lighted from a 1000 w. lamp the respiration rate actually showed distinct increase throughout the period that the leaf was in light. On removing the source of light the respiration rate fell off to assume the rate of respiration in darkness.

This rise in respiration, which is graphically represented by Fig. 5, can be due only to the catalysing action of light, for the

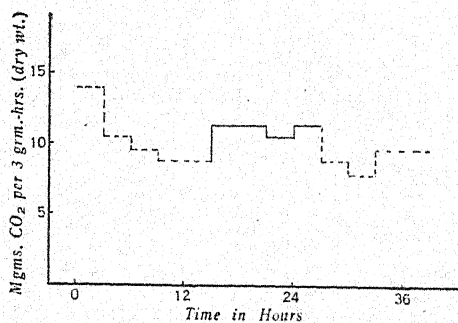


FIG. 5 ( $35^\circ \text{C}$ )

Respiration of non-green leaves of *Croton*

----- Respiration in dark

————— Respiration in light

primary photochemical effect is to activate the reacting molecules. These subsequently decompose or react with other molecules. The increased activation of the molecules naturally brings about an increase in the successive steps leading to the ultimate production of  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .

(b) *The Role of the Yellow Pigments :—*

One must note here that there was no increase in the respiration rate when roots were exposed to light. The reason being that the roots were unable to absorb the necessary radiant energy required for activation purposes. On the other hand the yellow pigments in the *Croton* leaves absorb the solar radiations for the utilization in the oxidative processes. It is thus possible that the rôle of the yellow pigments in a green leaf is only to help the maintenance of a high respiration rate in light. The yellow pigments absorb the chemically active rays, which may directly be responsible for the high respiration rate. Further work in the elucidation of this problem is in progress.

### 3. *The After-Effect of Light*

(a) *A Comparison of the After-Effect with Floating Respiration :—*

The study of Figs. 1, 2, 3 and 4 show that  $\text{CO}_2$  given out decreases rapidly in light due, obviously, to its being assimilated. We must remember however that the current of air previous to entering the leaf chamber is deprived of all the  $\text{CO}_2$ . So that the only assimilation in light is the assimilation of the respired  $\text{CO}_2$ . Thus towards the end of the light period, the maximum carbohydrate formed will be the amount broken down in respiration i.e., the carbohydrate at the end of light will be the same as at the beginning. Thus it is natural to suppose that the respiration rate at the end of light will start at the same plane as at the beginning. Fig. 6 shows the respiration values from light to darkness. On analysing it appears that :—

(1) The respiration rate in each case except "6 B" rapidly mounts up and reaches a maximum which is considerably above the respiration rate previous to giving light.

(2) The respiration rate in each case except "6 B" after reaching a maximum again rapidly climbs down.

From this one concludes that in light the respiration rate is high, but due to assimilation of the respired  $\text{CO}_2$  its true rate is masked. The high rate is noticed soon after light. In darkness, however, the respiration rate rapidly climbs down. Only by manipulations of the respiration curves backwards to the time when light was switched off, or to the zero hour of darkness, one arrives at the real initial respiration values in light. Due to complexities in the diffusion of  $\text{CO}_2$  and to the unavoidable experimental difficulties it is impossible to get at once the full effect of light. The curved lines marked A indicates the possible respiration rate in light and its downward path in darkness. A comparison of these

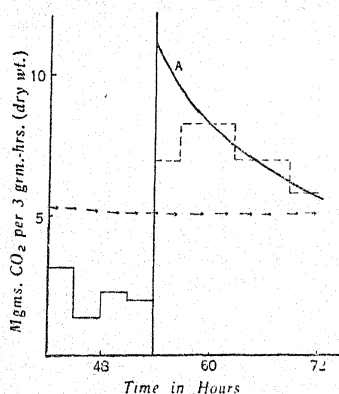


FIG. 6 A (35° C)

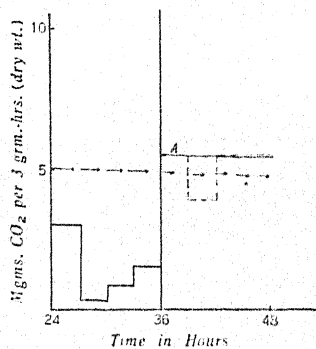


FIG. 6 B (20.5° C)

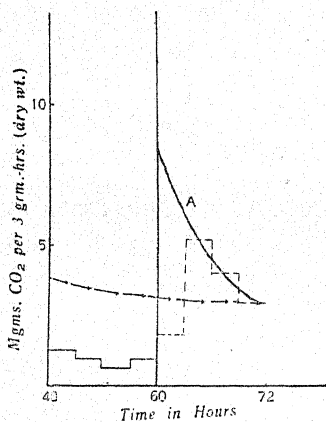


FIG. 6 C (27° C)

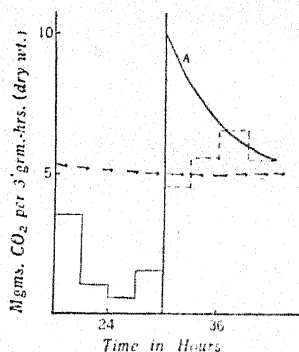


FIG. 6 D (40° C)

Explanation of Fig. 6 A, B, C, D

→ → → → Respiratory drift in dark

———— CO<sub>2</sub> given out in light

----- Respiration in dark

— A — Hypothetical after-effect of light

curves with the floating part of the curves in Figs. 1 and 2 shows a striking similarity of the two. In other words one may say that the respiration rate after light is really synonymous with "the floating part of respiration".

(b) Analytic Study of CO<sub>2</sub> Emission in Light :—

Coming to the actual CO<sub>2</sub> emission in light the preceding figures show that it drops to a minimum after some hours. Thereafter it augments slightly in light (see Fig. 6). This irrational behaviour, though at first appearing rather queer, is explained on

the assumption that the respiration rate is at a minimum in darkness. Naturally when light is supplied most of the  $\text{CO}_2$  of respiration gets assimilated, and thus less of it diffuses out. In a constant light the assimilation rate keeps constant. But however, in view of what has been said previously, respiration rate gradually increases in light so that more of  $\text{CO}_2$  remains unassimilated and this extra amount of  $\text{CO}_2$  will be given out.

#### 4. *The Respiration Rate, at Different Temperatures, in Light*

##### (a) *The After-Effect at Different Temperatures :—*

The study of the after-effect of light is the best way to find out the respiration rate of a green tissue in light. Fig. 6 gives the after-effects at different temperatures.

The hypothetical curves A drawn back to the hour when light was cut off show that there is a progressive relative increase of respiration in light over the respiration rate in darkness as temperatures decrease. Thus at  $40^\circ \text{C}$  it is 2, at  $35^\circ \text{C}$  2.2, at  $27^\circ \text{C}$  2.7. This however does not hold good at  $20^\circ \text{C}$  where there is practically no relative increase, the corresponding figure here being unity.

This change in the relative increase in respiration rate in the inverse proportion may be due to this that at higher temperatures the reacting molecules are already in an excited form; and as there is a limit to the number of molecules which can be thus excited, exposure to light has progressively lesser effect. Added to this, of course, at the high temperature of  $40^\circ \text{C}$  is the time factor which becomes also operative.

##### (b) *Light acts not as a Catalyst :—*

According to some, light acts as a catalyst on a photosensitive reaction because, in exoenergetic phenomena, light simply accelerates a process which otherwise goes on spontaneously in darkness. But light cannot act as a true catalyst, for a catalyst is not a source of energy. According to the law of Grotthus-Draper "In photochemical changes, light supplies the energy necessary for the activation of molecules". Thus when light acts upon a chemical system it always supplies energy to it. This is proved in the case of the respiration rates of yellow *Croton* leaves, which absorb light and this increases the respiration rate. The roots on the other hand are incapable of absorbing the radiations of the right type and so cannot increase their respiration rates.

##### (c) *The Respiration Rate in Light at $20.5^\circ \text{C}$ :—*

The respiration rate of leaves at  $20.5^\circ \text{C}$  is in many respects different from those at higher temperatures. The protoplasmic respiration keeps to the level of the protoplasmic respiration rates at  $35^\circ \text{C}$  and  $40^\circ \text{C}$  which temperatures are relatively high. This enhanced rate may be due to the altered starch-sugar equilibrium in favour of more sugar at lower temperature pointed out by many previous workers. The  $\text{CO}_2$  emission in light is somewhat like the

previous experiments in the sense that at the end of 3 hours it drops to a minimum and thereafter it progressively increases.

The after-effect of light, on the other hand, is different from the previous cases. The respiration rate quickly mounting up reaches a maximum of 5.5 mg. CO<sub>2</sub> which is maintained throughout the experiment. This respiration rate however is only slightly above the prelight value.

The slight increase of respiration and its maintenance at a constant rate shows that at this temperature light has very little effect upon it. Here the temperature is a distinctly limiting factor because the successive chains of reactions in respiration are governed by both light and temperature. But when the temperature is limiting light alone is incapable of activating the reactions.

#### SUMMARY

Experiments were carried out on the respiration of *Pistia* shoots and roots and the results have been reported.

An analysis of the data presented shows that light increases the rate of *Pistia* shoots and not of the roots. It has been pointed out that in the case of yellow *Croton* leaves also light has an accelerating effect on the respiration rate.

A study of the temperature effect on respiration in light shows that, along with a pure chemical or thermal stage, there is also a distinct photochemical stage in respiration in light. This is proved by the fact that at 20° C., where the temperature is limiting the rate of the reactions involved in respiration, light has practically no effect. Whereas at 27° C., when temperature no longer limits the reaction, there is maximum acceleration by light. Beyond this temperature the rate of increase progressively decreases. The acceleration of the respiration rate in light is due to the activation of the reacting molecules in light. In the case of roots, due to the absence of necessary pigments which can absorb radiant energy, there can be no photo-oxidation and hence there is no rise of the respiratory rate in light. The yellow pigments in the *Croton* leaves, on the other hand, absorb the solar radiations in the oxidative process and thus, possibly, help the maintenance of a high respiration rate in light.

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## STUDIES IN THE APOCYNACEÆ\*

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Received for publication on July 20, 1940

(Communicated by M. A. Sampathkumaran)

A STUDY of the existing literature as reviewed by Schnarf (1931) shows that the earliest reference to the family is found in the work of Hofmeister (1858). A few statements relating to the family are also found in the works of Vesque (1878 and 79), Strasburger (1884), Pitzorno (1891) and Billings (1901). The first detailed account of the life-history of an Apocynaceous plant, however, is that of Frye and Blodgett (1905) on *Apocynum androsaemifolium*. Since then important contributions to the literature on the family have been made by Guignard (1917 *a* and *b*) and Axel Andersson (1931). Andersson's work is the most comprehensive and, in a comparative account of the embryology of a number of plants, he has discussed the various aspects of their life-histories with reference to previous literature. An account of the development of microspores and the male gametophyte in species of *Vinca* is found in the works of Täckholm and Söderberg (1918) and Finn (1928).

Recently Schürhoff and Müller (1937) have published a cytological account of the haploid generations in some European species of Apocynaceæ; according to them the basic number of chromosomes for the family is 8. Meyer's (1938) account of the development of pollen and embryo-sacs in four species of Apocynaceæ is the most recent contribution to the literature on the family.

## MATERIALS AND METHODS

The present study was undertaken with a view to investigate some of the species available locally. Those selected for study are *Cerbera Odollam* Gärtn., *Vallaris Heyneii* Spreng., *Ichnocarpus frutescens* R.Br., *Wrightia tinctoria* R.Br., *Carissa carandas* Linn., and *Funtumia elastica* Stapf. The last named plant which yields the "Ire" Rubber" is a native of S. America and material was collected from a plant cultivated in the Government Botanical Gardens, Lal-Bagh, Bangalore.

The materials for study were collected on bright sunny days and killed in Bouin's fluid which gave satisfactory results. Dehydration and imbedding were done in the customary manner. Sections were cut at various thicknesses between 6 and 18 microns according to requirements. Heidenhain's iron alum hæmatoxylin was used for staining throughout the study.

\* Part of Thesis accepted for the Degree of Master of Science of the University of Mysore, 1938.



## THE FLOWER

The flowers are arranged in terminal or axillary cymes. The inflorescence may often be a fascicle as in *Vallisneria Heyneii*.

The origin and development of the floral parts take place in the usual manner. The sepals are the first to appear followed later by the petals and stamens. The two carpelled ovary is the last to appear.

## THE ANTHUR AND DEVELOPMENT OF MICROSPORES

The development of the anther and the formation of microspores have been studied in *Cerbera Odollam* and *Vallisneria Heyneii*.

In the young anther, by a division of the hypodermal archesporium an outer primary parietal layer and an inner sporogenous layer are formed. The tapetum is derived from the primary parietal layer. The tapetal cells later become conspicuous but remain uninucleate throughout. In the young anther wall the wall layers are two to three in number (Fig. 1). The endothecium is not well defined in the old anther and the tapetal cells are found to possess prominent vacuoles (Fig. 2) but when the microspores are separated the tapetal cells are completely disorganised.

The meiotic divisions in the microspore mother cells are normal and no irregularities have been observed (Figs. 5-9). At the metaphase twenty bivalents can be counted in *Cerbera Odollam* (Fig. 3) and ten in *Vallisneria Heyneii* (Fig. 4). No cell wall is formed between the two daughter nuclei after the first division and during the second division the two spindles may lie parallel or at right angles.

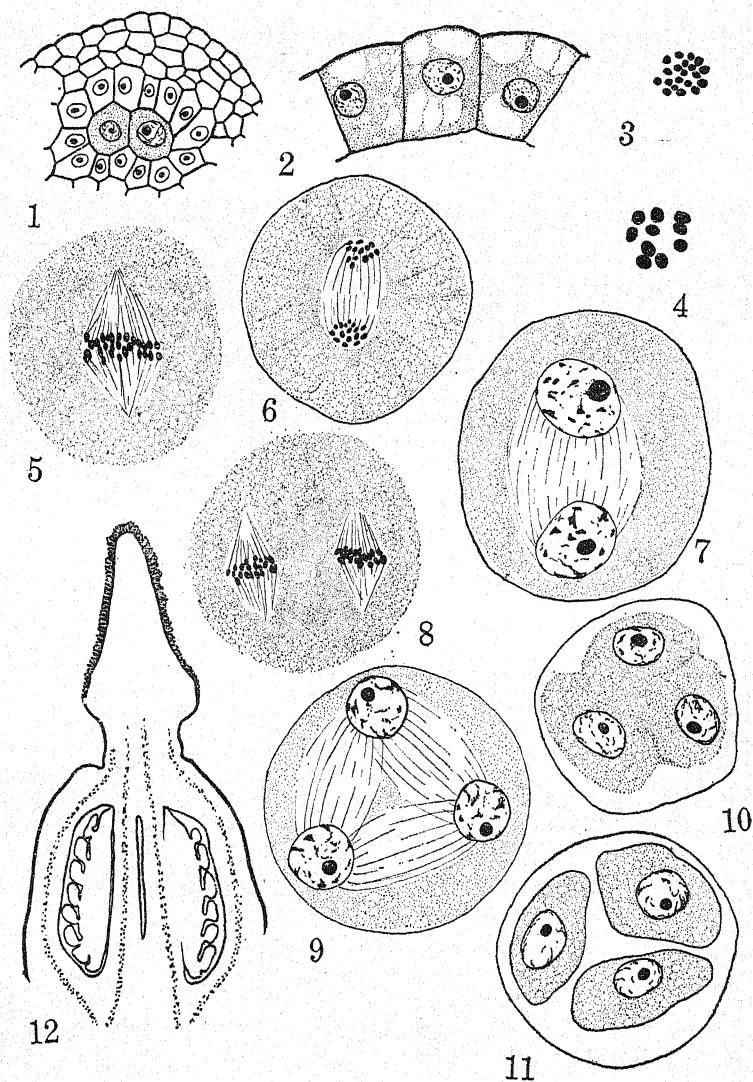
The separation of microspores takes place by the formation of peripheral furrows (Fig. 10) which gradually extend towards the centre and thus separate the microspores which are found lying free in the cavity of the original mother cell wall (Fig. 11).

Thus the development of microspores in the two plants studied follows the simultaneous type.

## DEVELOPMENT OF OVULE AND MEGASPOROGENESIS

The development of the ovule and the formation of the megaspores have been followed in detail in *Cerbera Odollam*, *Vallisneria Heyneii*, *Ichnocarpus frutescens*, *Wrightia tinctoria*, *Carissa carandas* and *Funtumia elastica*. The ovary is two carpelled in all and in *Cerbera Odollam*, the two carpels are free. A number of unicellular hairy outgrowths are seen on the stigma of *Wrightia tinctoria* (Fig. 12).

In the two carpelled ovary each ovule takes its origin on the thick placenta as a nucellar primordium and soon grows in size. The archesporium consisting usually of a single cell is differentiated early in the hypodermal layer of the nucellus (Figs. 13-15). The single integument takes its origin early and soon grows over and covers the nucellus. The archesporial cell does not cut off any



Figs. 1-12. Fig. 1. *Vallaris Heyneii*.—Section of young anther wall ( $\times 450$ ). Fig. 2. *Cerbera Odollam*.—Tapetal cells ( $\times 450$ ). Fig. 3. *C. Odollam*.—Metaphase plate ( $\times 1350$ ). Fig. 4. *V. Heyneii*.—Metaphase plate ( $\times 1350$ ). Figs. 5-8. *C. Odollam*.—Stages in meiosis ( $\times 1350$ ). Figs. 10 and 11. *C. Odollam*.—Separation of microspores ( $\times 900$ ). Fig. 12. *Wrightia tinctoria*.—Long. Section of ovary ( $\times 40$ ).

parietal cell but functions directly as the megaspore mother cell. The mother cell enlarges and is surrounded by a single epidermal layer of the nucellus (Fig. 14). The megaspore mother cell

undergoes the usual two divisions giving rise to a linear row of megaspores (Fig. 16). A linear row of megaspores is found in all the plants studied and this agrees with the previous observations of Guignard (1917) and Axel Andersson (1931).

The upper three megaspores degenerate in all cases and the chalazal megaspore develops into the embryo-sac (Figs. 17 and 18).

#### DEVELOPMENT OF THE EMBRYO-SAC

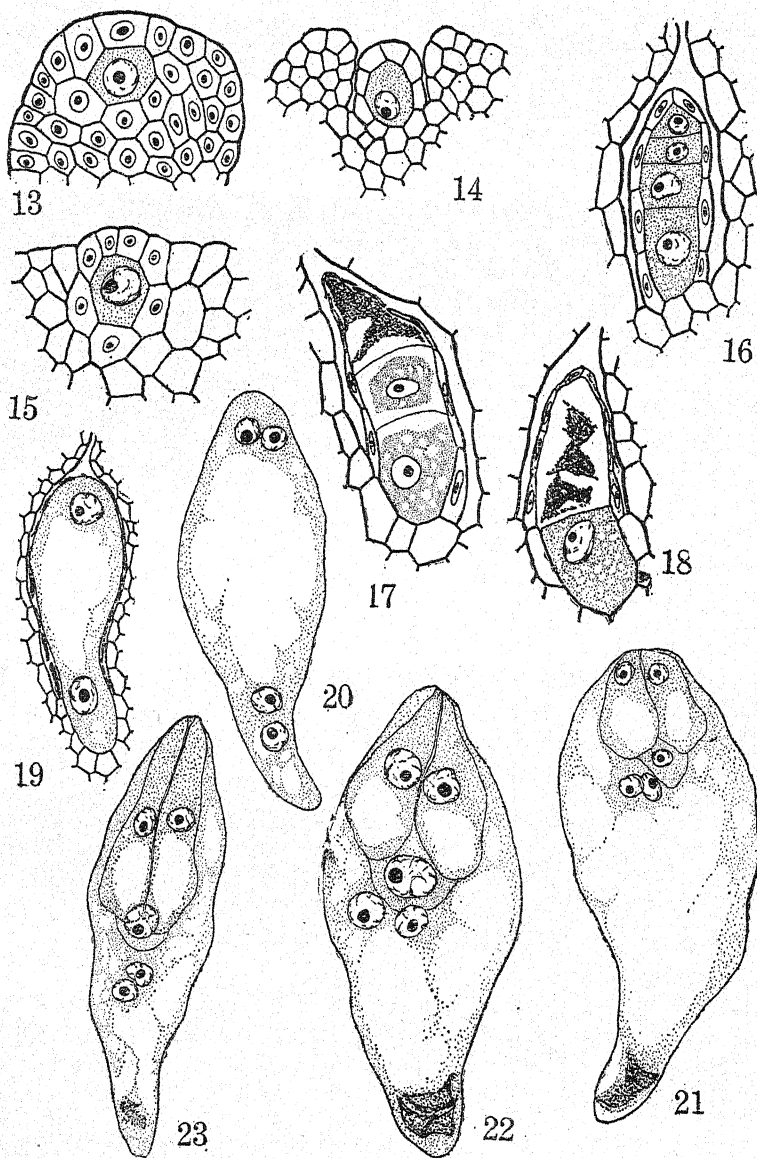
The development of the embryo-sac has been followed in all the six plants investigated. The development follows the usual type described for the majority of Angiosperms. The chalazal megaspore enlarges and its nucleus undergoes a division resulting in two nuclei which occupy the two poles of the sac (Fig. 19). A large vacuole is formed in the centre. The epidermal layer of the nucellus surrounding the embryo-sac generally breaks down at this stage though in *Funtumia elastica* and *Wrightia tinctoria*, the disintegration might start even earlier. This early disintegration of the nucellar epidermis has also been observed by Guignard (1917) who reports that in *Apocynum cannabinum* the nucellar epidermis breaks down when all the megaspores of the linear tetrad are still living.

The embryo-sac grows in size during the further stages. At the four-nucleate stage the remains of the disintegrated cells of the nucellar epidermis may be seen lining the embryo-sac as dark bits. The embryo-sac is now enclosed within the integument. The growing embryo-sac derives its nutrition from the cells of the integument adjoining the embryo-sac and also from the nucellar cells at the chalaza which are seen to possess rich contents. No distinct integumentary tapetum is formed in any of the plants investigated here. Billings (1901) and Léger (1913) reported that in *Amsonia salicifolia*, a distinct tapetum is formed by the innermost layer of the integument. Axel Andersson (1931) also reports a similar well-defined tapetum in *Rhazya orientalis* and *Amsonia tabernaemontana*.

While in all cases the micropylar end of the embryo-sac is found to be narrow and pointed, the chalazal end may be narrow and slightly bent as in *Cerbera Odollam*, or it may be broad as in *Ichnocarpus frutescens* and *Carissa carandas*.

The fully organised embryo-sac is of the typical eight-nucleate type with the egg apparatus, two polar nuclei and the three antipodals (Figs. 21-23). The egg apparatus consists of the two synergids and the egg. The synergids are very long in *Vallisneria Heynei* and *Carissa carandas*. In the former they almost reach the middle of the embryo-sac (Fig. 23). Such long synergids have been observed by Axel Andersson (1931) in *Ackocanthera spectabilis*. The synergids contain the usual basal vacuole.

The two polar nuclei from the two ends of the embryo-sac meet about half way and are first seen together at the middle of the sac. They migrate towards the micropylar end and finally



Figs. 13-23. Fig. 13. *Vallaris Heyneii*.—Archesporial cell. Fig. 14. *Cerbera Odollam*.—Megaspore mother cell. Fig. 15. *Ichnocarpus frutescens*.—Megaspore mother cell. Fig. 16. *Funtumia elastica*.—Linear tetrad of megaspores. Figs. 17 and 18. *Wrightia tinctoria*.—Degeneration of upper three megaspores. Fig. 19. *C. Odollam*.—Two nucleate embryo-sac. Fig. 20. *C. Odollam*.—Four nucleate embryo-sac. Figs. 21-23. Fully formed embryo-sacs of *C. Odollam*, *Wrightia tinctoria* and *Vallaris Heyneii* respectively. Figs. 13-18 ( $\times 900$ ). Figs. 19-21 ( $\times 400$ ). Figs. 22 and 23 ( $\times 900$ ).

occupy a position immediately below the egg. The polars fuse early in *Vallisneria Heyneii*, while in the others they are seen to be separate till a late stage and fuse just at the time of fertilisation. The fusion of the polar nuclei before fertilisation is comparatively rare among the plants studied by Axel Andersson (1931). According to him such a condition is seen only in isolated cases, as in *Allamanda nereifolia* and *Vinca minor*.

The antipodals are ephemeral in all the plants examined. Rarely do they remain conspicuous for a long time. In *Funtumia elastica* and *Wrightia tinctoria* they remain so for sometime but when the embryo-sac is ready for fertilisation they degenerate. Axel Andersson (1931) has also observed the early disorganisation of the antipodals in the majority of the plants he studied. Only in *Apocynum cannabinum* and *Rhazya orientalis*, he observed the presence of living antipodals in the embryo-sac at the time of fertilisation.

#### ABNORMAL OVULES

Though the presence of a single archesporial cell in the nucellus is the usual condition in the plants examined, instances were met with of ovules of *Cerbera Odollam* containing two megaspore mother cells, separated by a layer of nucellus (Fig. 24). Axel Andersson (1931) has also noted the presence of two megaspore mother cells, in an ovule of *Vinca minor*, but without a partition layer of nucellus. In another case were seen two linear tetrads of microspores separated by a layer of nucellus in *Cerbera Odollam* (Fig. 25).

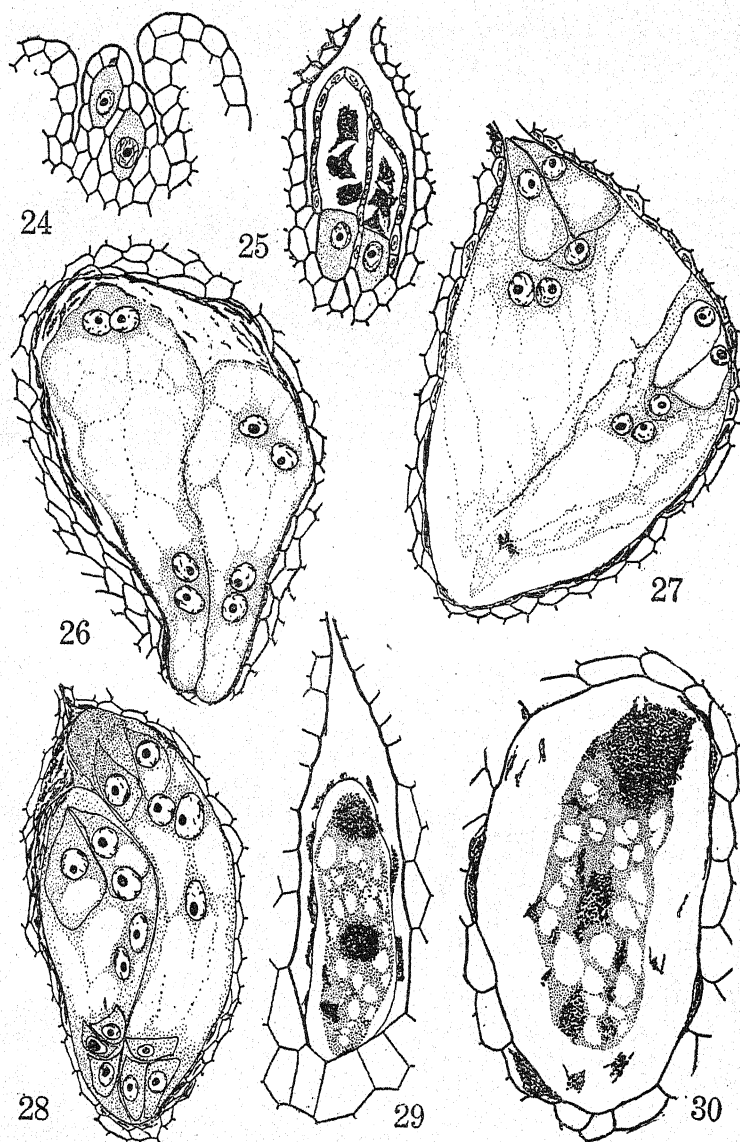
The presence of more than one embryo-sac in an ovule has been seen in the ovules of *Cerbera Odollam*, *Ichnocarpus frutescens* and *Wrightia tinctoria*. A large number of such ovules with accessory embryo-sacs were found in *Cerbera Odollam* (Figs. 26 and 27). Two fully developed sacs were met with sometimes (Figs. 27 and 28). It was not possible, however, to find the subsequent fate of such embryo-sacs. The entry of the pollen tube into one of the embryo-sacs was noticed in only one case in *Ichnocarpus frutescens*.

#### DEGENERATIONS IN THE EMBRYO-SAC

Degeneration of the embryo-sacs seems to be common in some of the plants studied (Figs. 29 and 30). Degeneration might set in at any stage of development. Fully organised embryo-sacs were seen to degenerate in *Vallisneria Heyneii* and *Ichnocarpus frutescens* (Fig. 30). The degeneration sets in first in the nucleus which gradually loses its outline and stains deeply. The cytoplasm along with the nucleus also takes a dark stain and shrinks away from the wall. In later stages of degeneration dark masses are seen distributed in the embryo-sac.

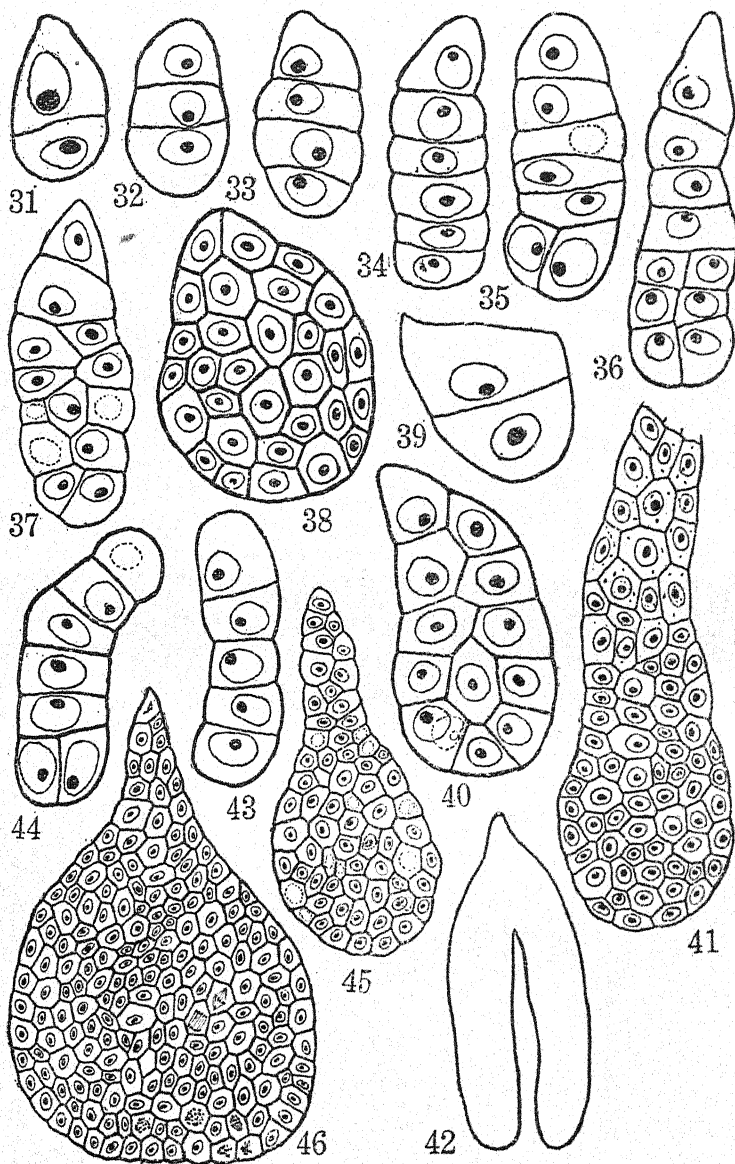
#### THE EMBRYO

The development of the embryo has been studied in *Cerbera Odollam*, *Carissa carandas*, and *Ichnocarpus frutescens*. *Vallisneria Heyneii* and *Funtumia elastica* are not known to produce seeds in Bangalore.



Figs. 24-27. *Cerbera Odollam*.—Fig. 24. Two megaspore mother cells; Fig. 25. Two tetrads; Fig. 26. Two four-nucleate embryo-sacs; and Fig. 27. Two organised embryo-sacs. Fig. 28. *Wrightia tinctoria*.—Two organised embryo-sacs. Fig. 29. *Vallaris Heyneii*.—Degenerating two-nucleate embryo-sac. Fig. 30. Degenerated fully formed embryo-sac. Figs. 24-27. ( $\times 400$ ). Figs. 28-30 ( $\times 900$ ).

The embryo-sac after fertilisation grows considerably in size. The fertilised egg undergoes a long period of rest and a large number



Figs. 31-46. Stages in the development of the embryo. Figs. 31-38. *Carissa carandas*. Figs. 39-42. *Cerbera Odollam*. Figs. 43-46. *Ichnocarpus frutescens*. Figs. 31-40 ( $\times 900$ ). Fig. 41 ( $\times 450$ ). Fig. 42 ( $\times 4$ ) (approximately). Figs. 43 and 44 ( $\times 900$ ). Figs. 45 and 46 ( $\times 400$ ).



of endosperm nuclei are formed by the time the first division takes place in the embryo. The first wall is transverse and a two-celled embryo results (Figs. 31 and 39). Further divisions in the embryo take place by transverse walls and thus a proembryo consisting usually of a row of six cells is formed (Figs. 32-35 and 43). The first cell of the six-celled proembryo divides by a vertical wall (Figs. 35 and 44) followed by vertical walls in the next two cells (Fig. 36). The further divisions are irregular and many oblique walls are laid (Figs. 37 and 40). This results in a massive embryo with a long suspensor. The basal cell disintegrates after a time. The suspensor is uniformly broad and in a longitudinal section appears three or four seriate (Fig. 41) in *Cerbera Odollam*. In *Ichnocarpus frutescens*, the suspensor is uni or biseriate and is also comparatively short (Figs. 45 and 46). The suspensor disintegrates in later stages.

The late embryo consists of a short radicle and the stem tip is present in a notch between the two thick cotyledons (Fig. 42).

#### THE ENDOSPERM

Endosperm formation begins long before the first division in the embryo takes place. A large number of free nuclei are formed and these arrange themselves peripherally in the embryo-sac. Wall formation begins in the endosperm when the embryo is three or four celled. Walls are first laid at the periphery and then towards the centre. In *Carissa carandas* and *Ichnocarpus frutescens* the embryo-sac, which is narrow and linear, is soon filled with endosperm cells. In later stages, due to extensive growth of the embryo-sac, a large cavity is formed in the centre. In *Cerbera Odollam* the embryo-sac is very broad and a cavity is present from the beginning. The endosperm tissue is extensive allround the embryo while it is only a thin layer of cells along the periphery of the embryo-sac.

#### CONCLUSIONS

The development of the microspores in the plants studied follows the simultaneous type. The same has been reported for *Vinca rosea* by Täckholm and Söderberg (1918) and for *Ackocanthera spectabilis* by Axel Andersson (1931). The simultaneous type is also noted in many of the species investigated recently by Schürhoff and Müller (1937) and by Meyer (1938). The successive type has been observed in some species studied by previous workers. As both the types are present in the plants belonging to the family no taxonomic significance can be attached for the occurrence of either mode of division. According to Meyer (1938), both the types are found in *Rauwolfia canescens*. She states, however, that the separation of microspores takes place by the formation of cell plates.

Regarding the occurrence of accessory embryo-sacs in an ovule the possibilities are that the extra embryo-sacs are derived either from megaspores belonging to different tetrads or from megaspores

belonging to the same tetrad. Numerous instances are recorded of the presence of multiple embryo-sacs in an ovule by previous investigators and such a feature is usually associated with a hybrid origin of the plant or it is due to polyploidy. The presence of accessory embryo-sacs, for example in *Alnus rugosa* (Woodworth, 1930) and in *Lychnis alba*  $\times$  *L. floscoculi* (Compton, 1912) are attributed to the hybrid origin of the plants concerned. Hurst (1931) found in certain diploid and polyploid species of *Rosa*, ovules with a number of embryo-sacs. No evolutionary significance can, however, be attached for the occurrence of this feature.

#### SUMMARY

The present investigation includes an embryological account of *Cerbera Odollam* Gaertn., *Vallaris Heyneii* Spreng., *Ichnocarpus frutescens* R.Br., *Wrightia tinctoria* R. Br., *Carissa carandas* Linn. and *Funtumia elastica* Stapf.

In the anther the hypodermal archesporium gives rise to the single layer of sporogenous cells and the primary parietal layer. The tapetum is derived from the latter.

Chromosome numbers have been determined for *Cerbera Odollam* and *Vallaris Heyneii*. The haploid numbers are 20 and 10 respectively. Separation of microspores follows the simultaneous type and is effected by quadripartition furrows.

A single archesporial cell in the hypodermal layer of the nucellus becomes directly the megaspore mother cell without cutting off any parietal cell. The chalazal megaspore of the linear tetrad develops into the embryo-sac.

The development of the embryo-sac follows the normal type. The single layer of nucellar epidermis disorganises early. No distinct tapetum is formed around the embryo-sac, but the cells of the integument and the chalazal nucellar cells supply nutrition.

Several abnormal ovules with accessory embryo-sacs have been recorded in *Cerbera Odollam*, *Ichnocarpus frutescens* and *Wrightia tinctoria*.

The embryo development is uniform in the three plants investigated. A six-celled proembryo is first formed which later differentiates into a suspensor and a massive embryo.

In the late embryo the radicle is short and the stem tip is present in a notch between the two cotyledons.

Endosperm is nuclear at the beginning, but cell formation takes place later and proceeds in a centripetal manner.

The writer wishes to express his gratitude to Dr. M. A. Sampathkumaran, Professor of Botany, University of Mysore, for kind guidance and encouragement during the progress of this work. He also wishes to express his appreciation of the aid extended by Mr. S. B. Kausik at various stages during the course of this study.

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# THE DEVELOPMENT OF THE EMBRYO-SAC AND FORMATION OF HAUSTORIA IN *LANTANA INDICA* ROXB., AND *STACHYTARPHETA INDICA* VAHL

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Received for publication on July 31, 1940

## INTRODUCTION

THE earliest embryological contributions on the family Verbenaceæ are by Treub (1883) and Hofmeister (1858), dealing with the development of the endosperm and haustoria in *Verbena officinalis*. Several other species of *Verbena* were studied by Kanda (1920) who reported a Helobiales-type of endosperm in that genus. Dahlgren (1923) is highly sceptical in regard to the accuracy of this statement and Schnarf's (1925) account of *Verbena officinalis* reveals that the endosperm is really cellular. According to Karsten (1891) the development of the embryo-sac in *Avicennia officinalis* is of the *Allium*-type, but Maheshwari (1937) feels that this needs reinvestigation. Schwencke (1931) observes that in the endosperm development of *Verbena* the sequence of wall-formation is variable and stresses the need for further work on tropical members of the family. More recently Junell (1934) and Patermann (1935) have made an extensive study of the family including several species of *Lantana* and *Stachytarpheta*.

## MATERIAL AND METHODS

The material was collected in fields near Bangalore and fixed on bright days between 10 a.m. and 2 p.m. Satisfactory results were obtained by using Bouin's fluid with 10% more of glacial acetic acid than is provided in the usual formula. Fixation in acetic alcohol with equal parts of absolute alcohol and glacial acetic acid gave fair results. Junell (1934) mentions that some difficulty was experienced during fixation. While the present material did not give any such trouble, difficulty was experienced in sectioning the fruits on account of their hard endocarp. The sections varied in thickness from  $8\ \mu$  to  $10\ \mu$  for the earlier stages and up to  $16\ \mu$  for the development of endosperm and embryo. All the sections were stained in Heidenhain's iron-alum hæmatoxylin.

### *Lantana indica* Roxb.

**EMBRYO-SAC.**—The ovary is bilocular with a single ovule in each locule. The nucellus arises as an outgrowth of cells on the

placenta and is provided with a single massive integument. The hypodermal archesporial cell functions directly as the megaspore-mother cell. A linear tetrad of megaspores is formed by the divisions of the mother cell and the chalazal megaspore develops into the embryo-sac, while the three upper ones degenerate (Fig. 2). The tendency for all the megaspores to develop further, as observed by Junell (1931, p. 36) in *Lantana Camara* and *L. involucrata*, was found to be very rare in my material.

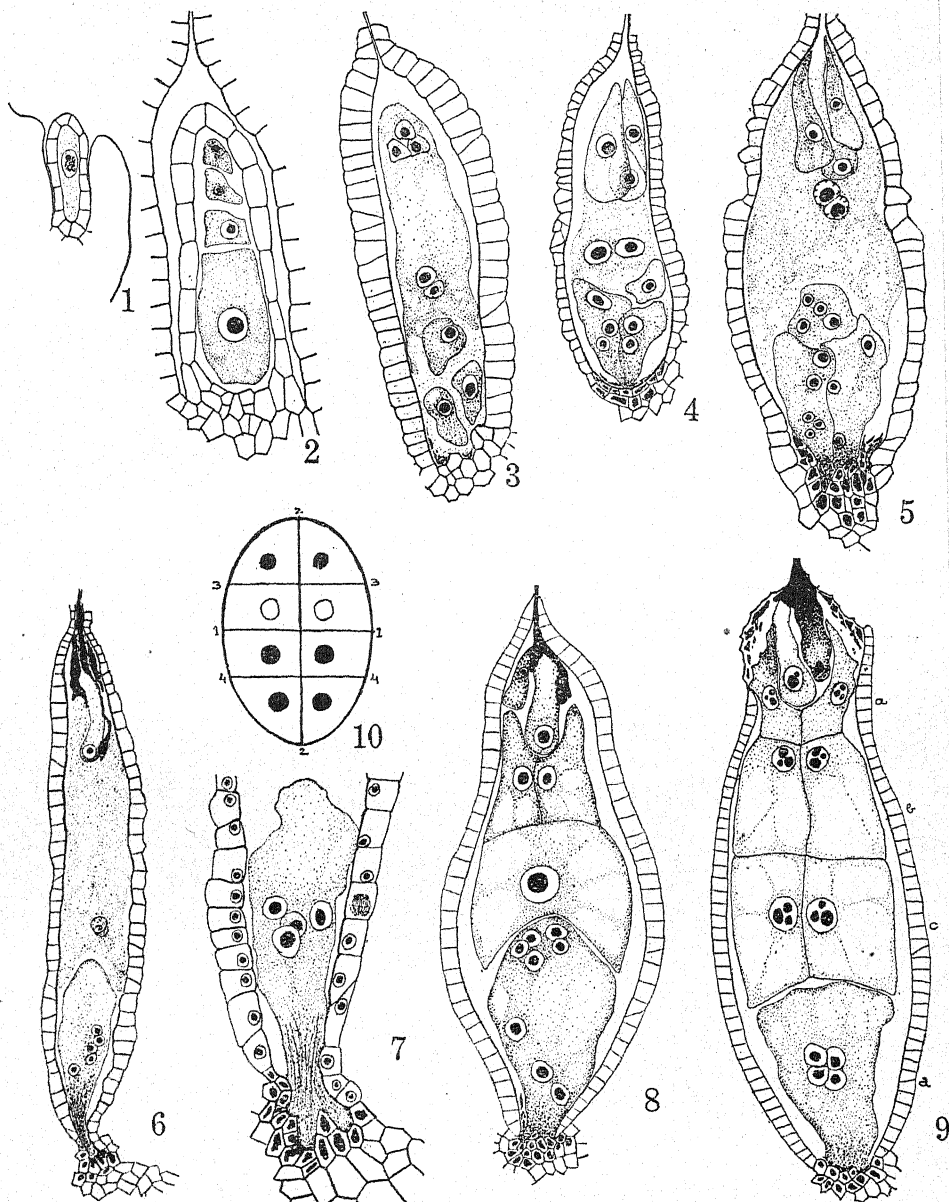
The functioning megaspore enlarges and further development proceeds along normal lines. From the four-nucleate condition onwards some of the cells at the chalazal region of the embryo-sac show a tendency to collapse and their contents take a dark stain. This appears to be due to the activity of the developing embryo-sac.

Even before the complete organisation of the egg-apparatus, the three antipodal cells increase in size and begin to take a deeper stain (Fig. 3). The nucleus undergoes some mitotic divisions so that each antipodal cell becomes three- to six-nucleate. As further enlargement proceeds, the chalazal end becomes tapering, and shows cytoplasmic striations similar to those seen in synergids, while a vacuole develops in the broad upper portion (Figs. 4 & 5). Thus, the three enlarged multinucleate antipodals together seem to form a very aggressive haustorium. Finger-like protrusions directed from them enter the chalazal tissue and their activity is clearly indicated by the degenerating cells of that region. At the time of fertilization there is a decrease in the number of nuclei in this antipodal apparatus owing to a degeneration of some of them (Fig. 6). The limiting membrane of each antipodal cell, which is clearly seen during the earlier stages also disappears at the time of fertilization. In the mature embryo-sac the two polar nuclei lie in close proximity, and at its chalazal end is seen the antipodal haustorium which persists till late at the time of seed formation (Fig. 12).

The embryo-sac as a whole is invested by the integumentary tapetum which shows its maximum activity just before the organisation of the antipodal haustorium (Fig. 3), and double fertilization takes place normally. The polar nuclei fuse just before fertilization (Fig. 6).

**EMBRYO.**—The divisions in the fertilised egg are delayed until the organisation of the haustoria and the development of the endosperm. The first division is transverse and separates the primary suspensor cell from the embryonal cell. By further divisions the former produces a long suspensor of six to eight cells which thrusts the embryo deep into the endosperm. The first and the second divisions in the embryonal cell are vertical and the third is transverse. The further development of the embryo is normal (Figs. 13–15).

**ENDOSPERM.**—The first division of the primary endosperm nucleus is followed by a transverse wall. The next division is longitudinal and takes place first in the micropylar chamber and later in the chalazal (Figs. 8 & 9). A transverse division in the two micropylar cells thus formed results in the separation of a micropylar tier



Figs. 1-10. *Lantana indica* Roxb.—Fig. 1. Megaspore-mother cell ( $\times 160$ ). Fig. 2. Linear tetrad of megaspores ( $\times 480$ ). Figs. 3-5. Stages in the organisation of the eight-nucleate embryo-sac and the formation of the antipodal haustorium ( $\times 365$ ). Fig. 6. Fertilization ( $\times 160$ ). Fig. 7. Enlarged view of the antipodal haustorium ( $\times 365$ ). Fig. 8. First and second divisions of the primary endosperm nucleus ( $\times 320$ ). Fig. 9. Development of the endosperm and differentiation of micropylar haustorium ( $\times 320$ ). (a) Micropylar haustorium, (b and c) Endosperm, (d) Antipodal haustorium. Fig. 10. Sequence of divisions in the primary endosperm nucleus (diagrammatic representation).



from the inner tier and it is from the latter that the endosperm develops (Fig. 10).

**HAUSTORIA.**—The micropylar tier develops into two uni-nucleate haustorial cells. These are simple and only mildly aggressive. This may perhaps be correlated with the organization of a very efficient haustorial apparatus at the other end of the embryo-sac (Figs. 8 & 9).

The chalazal cell separated by the first division of the primary endosperm nucleus gives rise by divisions to a mass of cells which connects the antipodal haustorium with the endosperm. These cells are smaller and stain more deeply than the endosperm cells above and have presumably a conducting function (Fig. 12).

*Stachytarpheta indica* Vahl

**EMBRYO-SAC.**—Megaspore formation and development of the embryo-sac take place normally. In the mature embryo-sac the two polar nuclei unite to form the secondary nucleus (Fig. 16). Soon after fertilization the three antipodals degenerate. There is a conspicuous tapetal jacket investing the embryo-sac. During fertilization one of the synergids is destroyed by the pollen tube entering the embryo-sac.

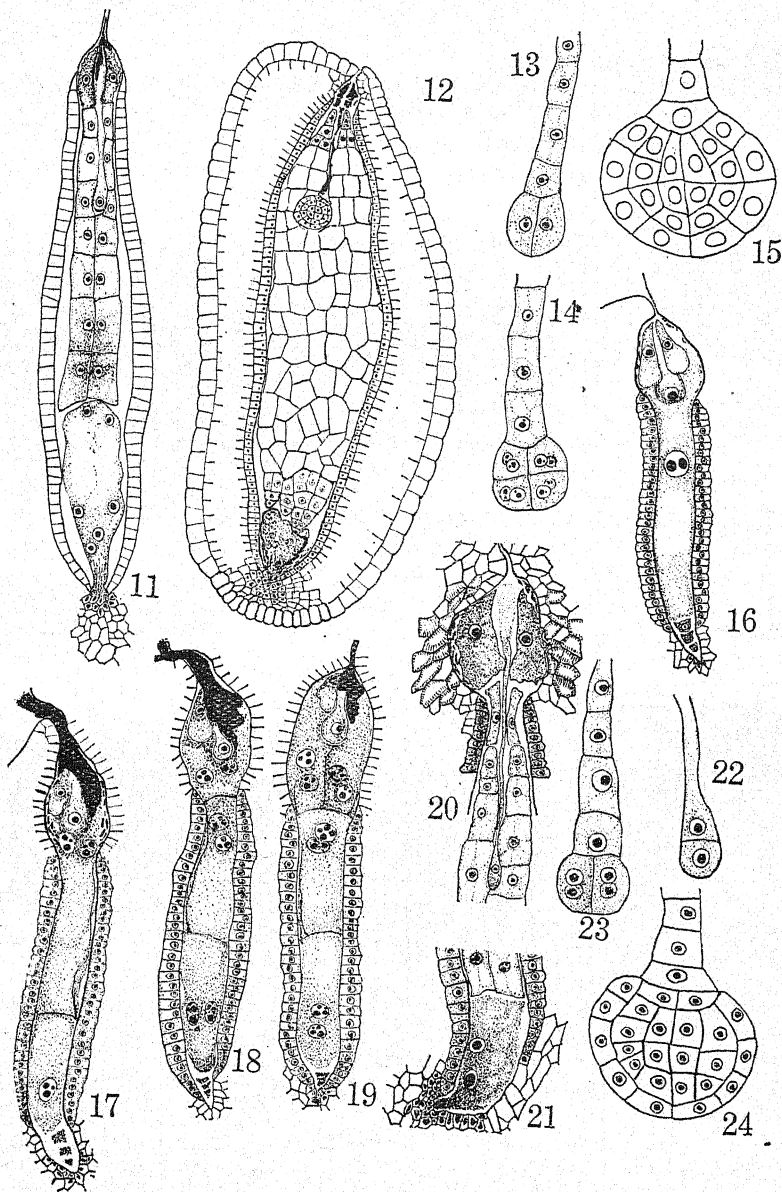
**EMBRYO.**—The fertilised egg divides after the endosperm haustoria and the endosperm are formed. The development of the embryo corresponds to the normal type (Figs. 22–24).

**ENDOSPERM.**—The first division of the primary endosperm nucleus followed by a transverse wall results in the formation of the micropylar and chalazal chambers. The micropylar chamber divides vertically giving rise to two daughter cells from the micropylar tier. This former gives rise to the endosperm (Figs. 17 & 18).

**HAUSTORIA.**—The two cells of the micropylar tier divide longitudinally and the four uni-nucleate cells form the micropylar haustoria (Fig. 19). These are very aggressive and crescent-shaped with their outer margins exhibiting a serrated appearance. Paternmann (1935) reports that in *Stachytarpheta cayennensis* and *S. indica* L., the micropylar haustorium is bi-nucleate, but this has not been observed in the local species. According to Junell (1934) the number of micropylar haustorial cells in *S. dichotoma* is four and in *S. angustifolia* it is greater, though the exact number is not specified.

The cells of the integument in the neighbourhood of the haustoria show reticulate thickenings of their walls, indicating a probable mechanical rôle (Fig. 20).

The nucleus of the chalazal chamber divides and a bi-nucleate uni-cellular haustorium is organised (Fig. 21). Such a bi-nucleate chalazal haustorium has also been observed in other species of *Stachytarpheta* by Paternmann (1935).



Figs. 11-15. *Lantana indica* Roxb. Fig. 11. L.S. showing the development of endosperm and the antipodal haustorium ( $\times 160$ ). Fig. 12. L.S. of an almost mature seed showing the embryo, endosperm and persistent antipodal haustorium ( $\times 65$ ). Figs. 13-15. Stages in the development of the embryo. Figs. 13 and 14 ( $\times 320$ ). Fig. 15 ( $\times 365$ ).

Figs. 16-24. *Stachytarpheta indica* Vahl.—Fig. 16. Mature embryo-sac ( $\times 160$ ). Fig. 17. First and second divisions of the primary endosperm nucleus ( $\times 160$ ). Figs. 18 and 19. Organisation of the chalazal and micropylar haustoria ( $\times 160$ ). Fig. 20. Micropylar haustorium ( $\times 160$ ). Fig. 21. Chalazal haustorium ( $\times 160$ ). Figs. 22-24. Stages in the development of the embryo. Figs. 22 and 23 ( $\times 320$ ). Fig. 24 ( $\times 365$ ).

## CONCLUSIONS

The variations in the development of haustoria observed in this family are briefly stated below :—

Patermann (1935) reports that in *Lantana trifolia* the three antipodals degenerate quite early. Though the earliest stage in the development of endosperm has not been observed, he finds that both the micropylar and chalazal haustoria are bi-nucleate. He also observes that while the micropylar haustorium is but feebly developed, the chalazal is very prominent and pierces the cells lying beneath it until it reaches the vascular traces of the funiculus.

In *Lantana indica* Roxb., the haustorium at the chalazal end of the embryo-sac is composed of three enlarged multi-nucleate antipodals. This is clearly indicated by the marked hypertrophy of these cells and the divisions of their nuclei even before the embryo-sac is mature. The fact that the chalazal haustorium is very conspicuous and already begins sending down processes even at the time of fertilization (Fig. 6), clearly indicates that it is not formed out of the endosperm as reported by Patermann, but from the antipodals.

The micropylar haustorium is of endospermal origin, but the two cells comprising it are uni-nucleate and not bi-nucleate as reported in other species.

A division of the antipodal cells with the consequent increase of their number has been reported by Misra (1939) in *Clerodendron phlomidis*. Junell (1931, p. 179) also mentions that several of his preparations of *Lantana* gave the impression of an increase in the activity of the antipodals but he believes such appearances to be due to the presence of some persisting megaspores. In many cases he saw as many as 8 nuclei lying at the chalazal end of the embryo-sac. An increase in the number of antipodal cells has also been reported by the same author in *Petraca volubilis*. But no mention is made by these two authors of the rôle of the antipodals in the formation of the haustorium. In *Lantana indica* there is no cell division but only nuclear division. At the time of fertilization there is a reduction in the number of nuclei owing to a degeneration of some of them; and the separating membranes between the cells disappear, resulting in a single large cell. A very aggressive and efficient nutritive organ is thus formed by the large persistent antipodal haustorium with its chalazal lobations. This type of haustorium has not been reported previously in Verbenaceæ, although it is a common feature in members of the Compositæ. The chalazal chamber separated by the first division of the primary endosperm nucleus does not develop into the usual chalazal haustorium but merely gives rise to a tissue concerned with transportation of nutritive materials from the haustorium to the endosperm. In *Stachytarpheta* conditions are different, for here the antipodals degenerate and it is the endosperm that is concerned with the formation of the bi-nucleate chalazal haustorium, as in many members belonging to the tribe Rhinanthæ of the Scrophularinæ (Schmid, 1906).

## SUMMARY

1. In both *Lantana indica* Roxb., and *Stachytarpheta indica* Vahl., the ovule is anatropous and is provided with a single massive integument.

2. Megasporogenesis proceeds normally. Occasionally all the megaspores begin to develop in *Lantana indica*.

3. In *Stachytarpheta indica*, the antipodals are ephemeral and degenerate shortly after fertilization, while in *L. indica* they become multi-nucleate and form an aggressive haustorium which sends down outgrowths into the chalazal tissue.

4. In *Lantana* the micropylar haustorium is two-celled and the chalazal chamber contributes towards the formation of cells connecting the antipodal haustorium with the endosperm. In *Stachytarpheta indica* the micropylar haustorium is composed of four uni-nucleate cells and the chalazal haustorium is bi-nucleate.

5. The development of the embryo is of the normal type in both *Lantana indica* and *Stachytarpheta indica*.

My grateful thanks are due to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M. (Chicago), and Mr. C. V. Krishna Iyengar, M.Sc., under whose guidance this work has been carried out. I am indebted to Dr. P. Maheshwari, D.Sc., F.N.I., of the Dacca University, for literature and kind criticism.

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# GAMETOGENESIS AND EMBRYOGENY IN FIVE SPECIES OF THE CONVOLVULACEÆ\*

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(Communicated by M. A. Sampathkumaran)

Received for publication on July 20, 1940

## CONTENTS

	PAGE
1. Introduction .. .. .	53
2. Materials and Methods .. .. .	54
3. Organogeny .. .. .	55
4. Microsporogenesis ( <i>Ipomoea Learii</i> and <i>Ipomoea staphylina</i> )	55
5. Megasporogenesis ( <i>Ipomoea Learii</i> , <i>Ipomoea staphylina</i> , <i>Ipomoea hederacea</i> , <i>Argyrea speciosa</i> and <i>Evolvulus alsinoides</i> ) .. .. .	59
6. Fertilization .. .. .	62
7. Endosperm .. .. .	62
8. Embryogeny .. .. .	62
9. Discussion .. .. .	63
10. Conclusion .. .. .	66
11. Summary .. .. .	66
12. Acknowledgments .. .. .	67
13. Bibliography .. .. .	68

## INTRODUCTION

THE family Convolvulaceæ consists of 15 genera and 800 species, mostly twiners, occurring in the Tropics (Bentham and Hooker).

The family has attracted the attention of plant morphologists and physiologists from a long time, on account of the peculiarities of nutrition in the parasitic species, as well as on account of other interesting features of development.

The earliest investigator to study the structure of the pollen grains in the family was Strasburger (1899), who described the pollen grains of the Convolvulaceæ to be spinescent. According to him, the structure of the pollen grains is after the manner of the Malvaceous type of construction.

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\*Thesis submitted in partial fulfilment of the requirements for the M.Sc. Degree of the Mysore University, 1937.

Peters (1908) reported the presence of parietal cells in the nucellus of the ovules of *Convolvulus* and *Cuscuta*, and this was seriously questioned by Dahlgren (1927).

Beer (1911), in the course of his researches on spore development in various plants, described the structure of the pollen grains of *Ipomoea purpurea*, and concluded that Strasburger's comparison was only superficial. According to Beer, the pollen grains of *Ipomoea purpurea* are spinescent with many exit pores. He described the origin and growth of the spines in the thick exine from the angles of a mesh-work of thickening bands.

Macpherson (1921) studied the embryogeny of *Cuscuta* and *Convolvulus* and found polyembryony in *Convolvulus* and the absence of cotyledonary development in *Cuscuta*. She reported the first division of the fertilized egg to be transverse, further divisions giving rise to a large suspensor.

Smith (1934) investigated the morphology and embryogeny in five species of *Cuscuta*. In *Cuscuta arvensis*, he reported the first division of the fertilized egg to be transverse and the second division to be perpendicular to the first and occurring only in the basal cell.

Mathur (1934) reported the presence of parietal cells in *Convolvulus arvensis*.

Datta (1936) reports a massive suspensor in *Convolvulus tricolor*. The sequence of divisions in the proembryo is not adequate in Datta's account of *Convolvulus tricolor*.

From this brief enumeration of the history of the subject, it is clear that there is a lot of conflicting opinion regarding the presence of parietal cells in the *Convolvulaceae*. Information regarding embryogeny is also incomplete. Besides, the cytological aspect has received scant attention.

The present investigation was, therefore, undertaken with a view to trace the course of sporogenesis, development of the gametophytes, embryogeny and cytology of five species of the *Convolvulaceae*.

#### MATERIALS AND METHODS

1. *Ipomoea Learii*
2. *Ipomoea hederacea*
3. *Ipomoea staphylinia*
4. *Argyreia speciosa*
5. *Evolvulus alsinoides*

These plants range from the giant creeper, *Argyreia speciosa*, to the small spreading herb, *Evolvulus alsinoides*, the rest being intermediate in size. These are all found to flower for the major part of the year except *Argyreia speciosa*, which has a limited flowering period.

Considerable difficulty was experienced during fixation due to the presence of latex in the tissues. A variety of fixatives was tried,



and the most satisfactory results were obtained with Bouin's fluid. Collections of the material were done between 10 A.M., and 3 P.M. during the months of July, August, September and October on bright warm sunny days. The dehydration, clearing and embedding were done in the usual manner.

Sections were cut at various thicknesses ranging from six to twenty microns, and stained with one per cent Heidenhain's iron alum hæmatoxylin, without any counter stain.

#### ORGANOGENY

1. *Ipomoea Learii*
2. *Ipomoea staphylina*
3. *Evolvulus alsinoides*

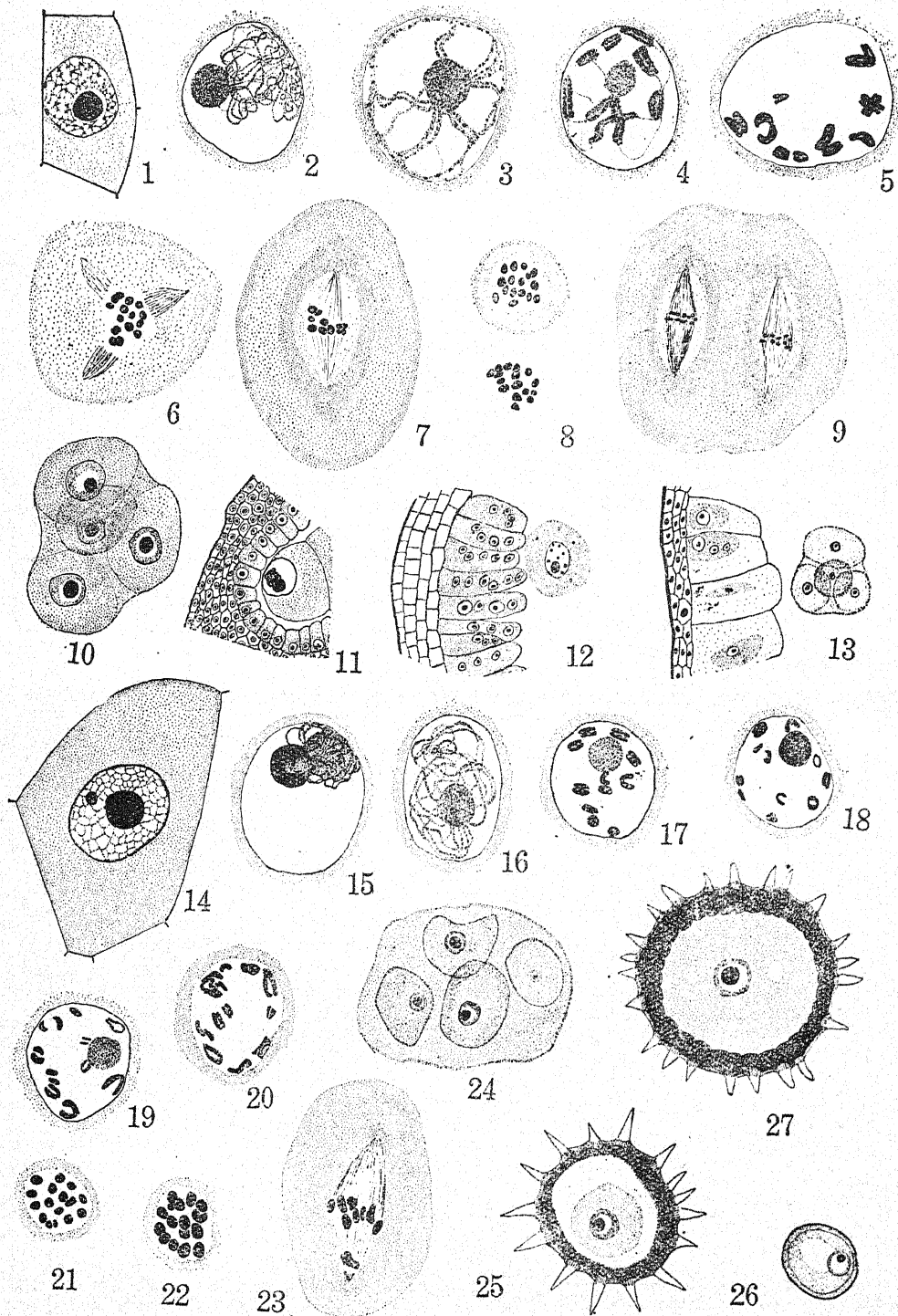
At an early stage, the rudiments of the flower make their appearances as protuberances in the axil of a single bract. The floral organs develop centripetally, the order of appearance being Calyx, Corolla, Andrœcium and Gynœcium.

#### MICROSPOROGENESIS

1. *Ipomoea Learii*
2. *Ipomoea staphylina*

The young anther is a homogeneous mass of small cells enclosed by means of an epidermis. It becomes fore-lobed early and corresponding to each lobe, a plate of hypodermal archesporial cells is differentiated from the surrounding cells. The archesporial cells are recognizable from the remaining cells by their dense cytoplasm, large size and affinity for stains. The archesporium undergoes a periclinal division giving rise to a primary parietal layer outside and a primary sporogenous layer inside. Subsequent periclinal divisions result in the formation of 3-5 layers of wall cells, in both *Ipomoea Learii* and *Ipomoea staphylina*. The outermost wall layer becomes the endothecium, while the innermost by elongation becomes the tapetum. Subsequent periclinal divisions result in the formation of four layers of wall cells. The middle layers lose their protoplasmic contents and become compressed at the time of the separation of the pollen grains. The tapetum becomes very conspicuous when the pollen mother cells are in the post-synizetic stages. During this time the tapetal cells enlarge and become multi-nucleate amitotically. Still later, when the tetrads are found enlarging, the tapetum consists of a layer of large cells of scanty cytoplasmic contents lining the walls of the loculi.

*Heterotypic mitosis in the pollen mother cells.*—The primary sporogenous cells enlarge greatly. They contain usually a single row of cells, but occasionally 2-3 layers are seen. The pollen mother cells are polygonal in shape, and the resting nucleus shows numerous chromatin threads which stain faintly (Figs. 1 and 14). Gradually the leptotene threads arrange themselves by parallel approximation. The nucleus shows a large nucleolus and one or more



Figs. 1-27. *Microsporogenesis*: Figs. 1-13. *Ipomoea Learii*. Fig. 1. Resting nucleus ( $\times 1600$ ). Fig. 2. Open spireme ( $\times 2700$ ). Fig. 3. Pachynema ( $\times 2700$ ). Fig. 4. Late diplonema ( $\times 3600$ ). Fig. 5. Diakinesis ( $\times 3600$ ). Fig. 6. Tripolar spindle ( $\times 3600$ ). Fig. 7. Metaphase

smaller ones. The parallelism of threads becomes more evident during zygonema. Following zygonema, there is a gradual contraction of the chromatin threads away from the nuclear membrane resulting in the synizetic knot. The large nucleolus is not involved in this contraction, while the smaller nucleolus seems to be included in the synizetic knot (Fig. 15). During synizesis the tapetal cells enlarge and contain dense cytoplasm (Fig. 11). Recovery from synizesis results in the formation of looped pachynema threads which are found to be thick and distinctly double (Figs. 2, 3 and 16). The gradual shortening and twisting of the threads gives rise to the strepsinema stage. When the diplotene threads are organized, the nucleolus loses its stainable reactions to some extent. During diplonema the chiasmata become less in number due to terminalization (Fig. 4). At the close of diakinesis the nuclear membrane shows signs of fading and the nucleolus disappears (Figs. 5 and 20). In the case of *Ipomoea Learii* fine threads are observed between the chromosome pairs.

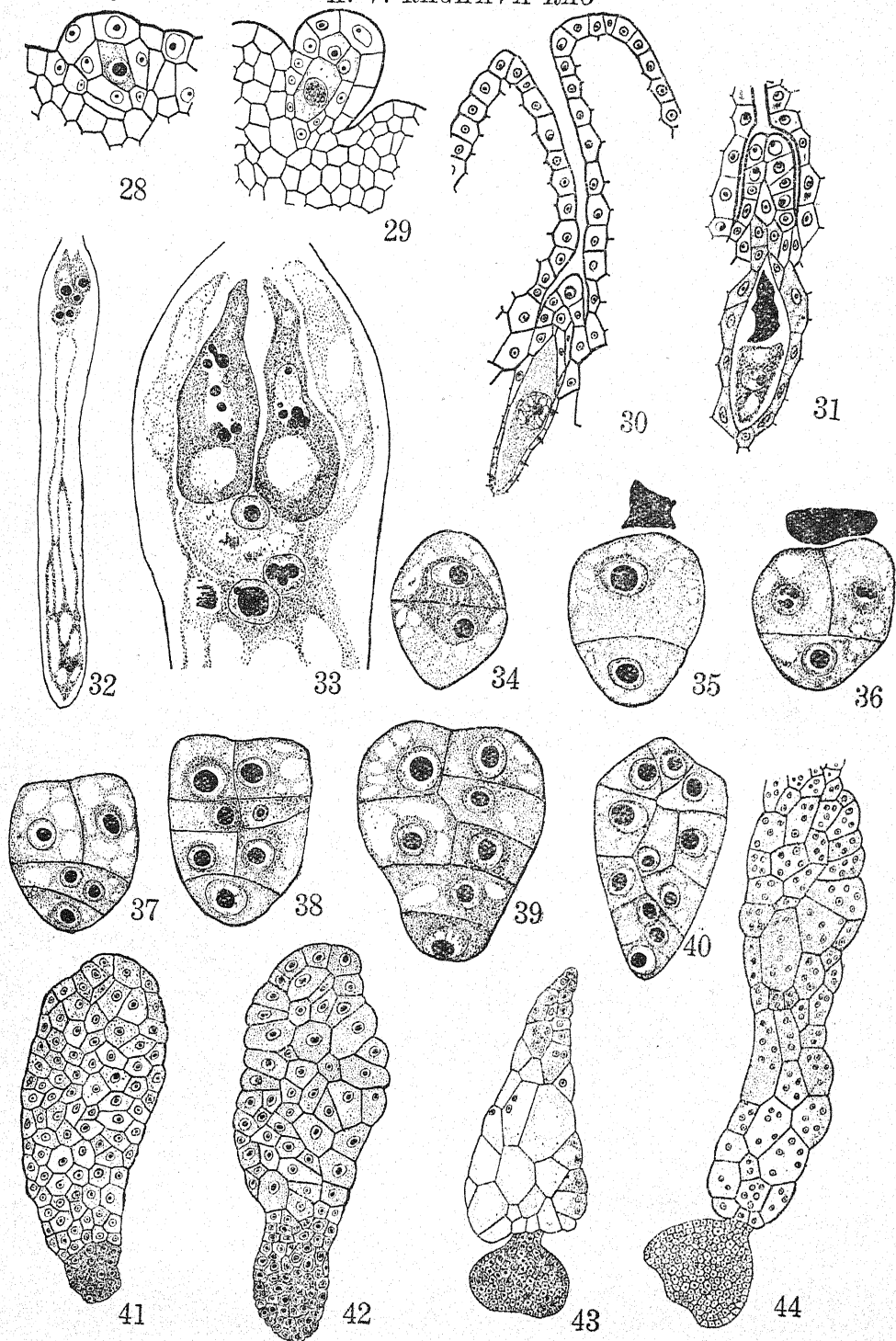
At this stage the tapetal cells are found to have 5-7 nuclei (Fig. 12). Some of the chromosomes in the mother cells are observed as univalents. This de-conjugation of the homologues is observed more frequently in the case of *Ipomoea staphylina* (Figs. 17, 18, 19 and 20). Difference in size and form of the bivalents are pronounced in *Ipomoea staphylina*. Some are longer than the others. Frequent occurrence of non-pairing and precocity are presumably responsible for the subsequent sterility of the pollen grains in this plant.

With the disappearance of the nuclear membrane a multipolar spindle is organised. It successively becomes tripolar and bipolar (Figs. 6 and 7). The bivalents are arranged at the equator of the bipolar spindle at the metaphase. In both *Ipomoea Learii* and *Ipomoea staphylina* sixteen bivalents are recognised (Figs. 8, 21 and 22). The univalents separate during anaphase and reach the poles in the normal way. In some cases precocity is observed (Fig. 23).

During the meta- and anaphases, these spindles are invested by a dense perinuclear mantle of granulated cytoplasm in *Ipomoea Learii* (Fig. 7). Such a mantle is not observed in *Ipomoea staphylina* (Fig. 23). After the telophase the daughter nuclei are reconstructed. There is a short interphase, at the end of which the homotypic division begins. The spindles of the second division are either

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of 1st division showing the perinuclear zone ( $\times 2700$ ). Fig. 8a. Metaphase plate (1st division) ( $\times 3600$ ). Fig. 8b. Metaphase plate (1st division) ( $\times 3600$ ). Fig. 9. Two metaphase spindles ( $\times 2700$ ). Fig. 10. Tetrahedral arrangement ( $\times 1260$ ). Fig. 11. Tapetal layer ( $\times 800$ ). Fig. 12. Multi-nucleate tapetal cells ( $\times 100$ ). Fig. 13. Compressed wall layers and developed tapetum ( $\times 800$ ). Figs. 14-27. *Ipomoea staphylina*. Fig. 14. A single microspore mother cell enlarged. Fig. 15. Synizesis ( $\times 2700$ ). Fig. 16. Opening of the synezetic knot ( $\times 2700$ ). Figs. 17, 18, 19 and 20. Diakinetic stages ( $\times 2700$ ). Figs. 21 and 22. Polar view of metaphase plate ( $\times 2700$ ). Fig. 23. Metaphase with precocious chromosome. Fig. 24. The pollen tetrad ( $\times 1800$ ). Fig. 25. Pollen grain of *Ipomoea staphylina* ( $\times 1200$ ). Fig. 26. Pollen grain of *Evolvulus alsinoides* ( $\times 1600$ ). Fig. 27. Pollen grain of *Argyreia speciosa* ( $\times 800$ ).



Figs. 28-44. Megasporogenesis and Embryogeny: *Ipomoea Learii*.  
 Fig. 28. Archesporial cell ( $\times 1260$ ). Fig. 29. Megaspore mother cell and

parallel to each other (quadrate) are at right angles (decussate). Granular and cytoplasmic zones are observed to invest the homotypic spindles too in *Ipomoea Learii* (Fig. 9). The chromosomes after reaching the poles reconstruct four daughter nuclei (Figs. 10 and 24). The microspores separate by furrowing. They are arranged in the tetrahedral shape in *Ipomoea Learii*, while the diagonal arrangement occurs in *Ipomoea staphylina* (Figs. 10 and 24).

In the early stages of pollen grains the cytoplasm is observed in two distinct zones, an outer dense zone and an inner light zone. The outer zone presumably develops the thick and hard exine. The thick exine forms a mesh-work of apertures. At the angles of these meshes spines develop (Fig. 25). The intine is a very thin membrane. The numerous apertures serve as exits for the pollen tube at the time of germination on the stigma.

The pollen grains of *Argyreia speciosa* (Fig. 27) are of exactly the same structure with blunt short spines, but are very much larger in size than the pollen grains of *Ipomoea*. In *Evolvulus* the pollen grain is considerably smaller than the pollen grains in the other two genera, with a smooth exine (Fig. 26). The ratio of the sizes of the pollen grains of *Evolvulus*, *Ipomoea* and *Argyreia*, when calculated, approximates to:—1:7:32 respectively. At the time of pollen dispersal, rarely three and commonly two nuclei are observed in the pollen grains.

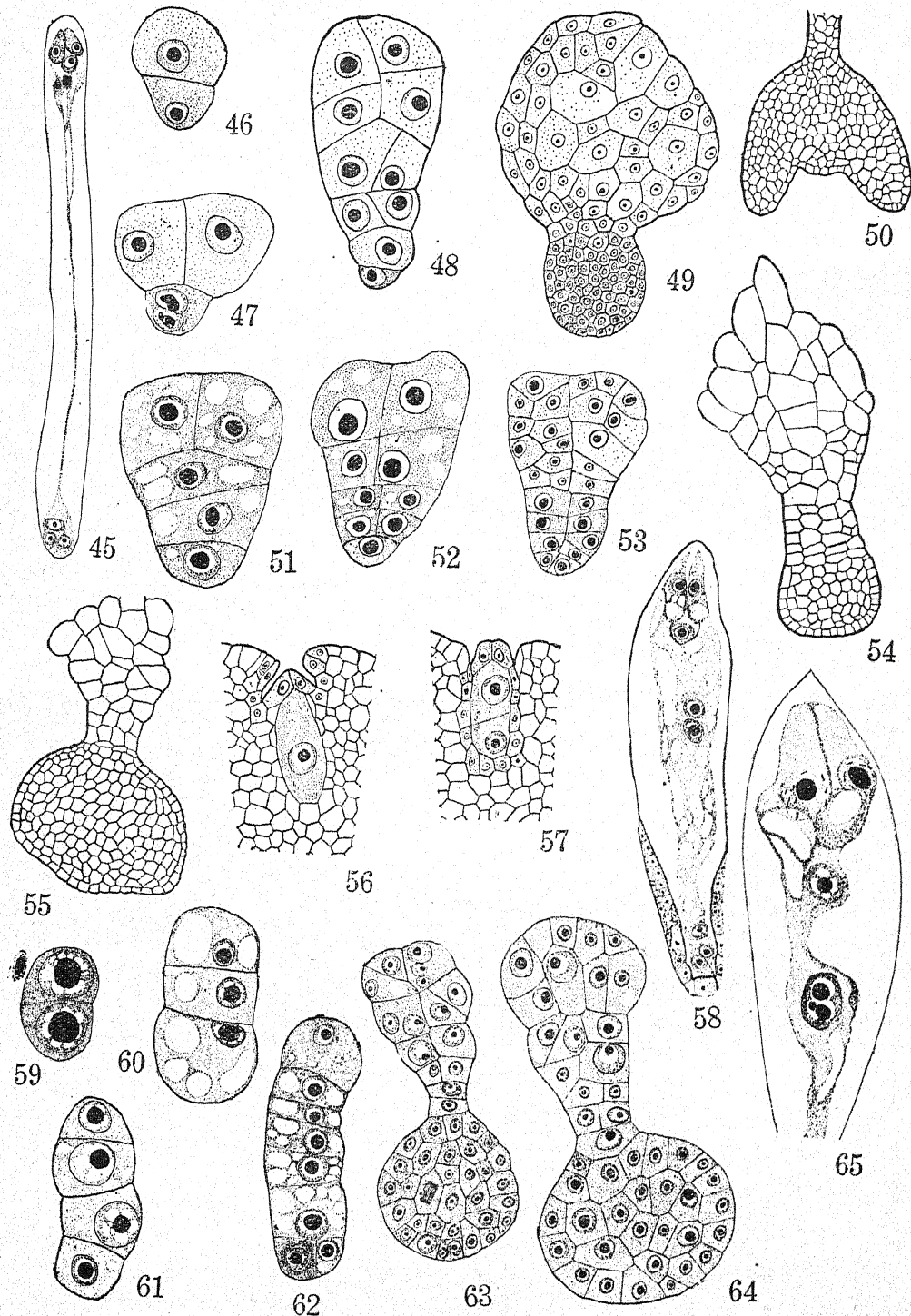
#### MEGASPOROGENESIS

1. *Ipomoea Learii*
2. *Ipomoea staphylina*
3. *Ipomoea hederacea*
4. *Argyreia speciosa*
5. *Evolvulus alsinoides*

The primordium of the ovule makes its appearance as a small knob-like protuberance. This increases in size by multiplication of the cells and becomes the nucellus of the ovule. By the time the nucellus becomes well differentiated, an annular growth begins to develop giving rise to the primordium of the single integument. The integument increases in size, becomes massive and over-arches the nucellus. The archesporium consists of a single cell, which is hypodermal and easily recognisable by its staining reactions, form and size, and by its nucleus (Fig. 28). The archesporial cell in *Ipomoea Learii* enlarges and by a transverse division contributes to

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parietal cells ( $\times 800$ ). Fig. 30. Deep-seated megaspore mother cell ( $\times 800$ ). Fig. 31. Chalazal megaspore enlarging ( $\times 900$ ). Fig. 32. Eight-nucleate embryo-sac ( $\times 560$ ). Fig. 33. Enlarged synergids with nucleolar budding ( $\times 1200$ ). Fig. 34. Two-celled pro-embryo ( $\times 1200$ ). Fig. 35. Two-celled pro-embryo ( $\times 1800$ ). Fig. 36. Three-celled pro-embryo ( $\times 1800$ ). Fig. 37. Pro-embryo ( $\times 1200$ ). Fig. 38. Pro-embryo ( $\times 1200$ ). Fig. 39. Pro-embryo ( $\times 1200$ ). Fig. 40. Pro-embryo, later stages ( $\times 1200$ ). Fig. 41. Embryo ( $\times 560$ ). Fig. 42. Embryo ( $\times 560$ ). Fig. 43. Embryo and suspensor ( $\times 240$ ). Fig. 44. Embryo and suspensor ( $\times 240$ ).



Figs. 45-65. *Megasporogenesis and Embryogeny*: Figs. 45-50. *Ipomoea hederacea*. Fig. 45. Eight-nucleate embryo-sac ( $\times 560$ ). Fig. 46. Pro-embryo (two-celled) ( $\times 1200$ ). Fig. 47. Four-celled embryo ( $\times 1200$ ).

the formation of an upper primary parietal cell and a lower primary sporogenous cell. The primary parietal cell first divides radially into two (Fig. 29) and then again anticlinally and periclinally (Figs. 30 and 31).

The sporogenous cell which functions as the megaspore mother cell becomes deep seated owing to the divisions and growth of the parietal cells (Fig. 31). The Sympetalæ are in general characterised by the absence of parietal cell formation, and it is very interesting to note the presence of the parietal tissue in *Ipomoea Learii*.

In *Ipomoea staphylina* the archesporial cell is hypodermal in origin and functions directly as the megaspore mother cell, without cutting off any parietal cells. In the case of *Evolvulus alsinoides* also no parietal cells are present (Figs. 56 and 57).

The megaspore mother cell undergoes the usual reduction division and gives rise to a linear tetrad of megaspores.

The chalazal megaspore increases in size and develops into the embryo-sac, while the upper megaspores degenerate.

*Female gametophyte*.—The functioning megaspore, by three successive mitotic divisions, gives rise to the normal eight-nucleate embryo-sac (Figs. 32, 45, 58 and 65). The embryo-sac at the time of maturity is found to be greatly elongated occupying almost the entire length of the ovule.

The egg-apparatus and polars are organised at the micropylar end in the normal way. The three antipodals degenerate very early. Only in the case of *Evolvulus* they are prominent (Fig. 58).

It is observed that the development of the embryo-sac is completed long before the flower buds open. By the time the flower buds open, the egg-apparatus and polars are found to have considerably enlarged. The synergids project towards the micropyle in the form of a beak-like extension. An interesting feature regarding the synergids is the nucleolar budding in *Ipomoea Learii* (Fig. 33).

In *Ipomoea Learii* the nuclei of the synergids are 2-3 times larger than the egg-nucleus, even from the beginning. In *Ipomoea staphylina*, the synergid-nuclei presumably resolve into chromosomes, before the contents of the embryo-sac abort entirely (Fig. 65).

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Fig. 48. Embryo ( $\times 1200$ ). Fig. 49. Embryo with massive suspensor ( $\times 500$ ). Fig. 50. Section of the embryo with cotyledonary differentiation ( $\times 240$ ). Figs. 51-55. *Argyrea speciosa*.—Fig. 51. Embryo suspensor cell divided vertically. Fig. 52. Embryo. Fig. 53. The suspensor has developed much and consists of many cells. Fig. 54. Embryo. Fig. 55. The same enlarged ( $\times 400$ ). Figs. 56-65. *Evolvulus alsinoides*. Fig. 56. Archesporial cell and integument initials ( $\times 1200$ ). Fig. 57. Dyad of the megaspore ( $\times 1200$ ). Fig. 58. Eight-nucleate embryo-sac ( $\times 900$ ). Fig. 59. Pro-embryo (two-celled) ( $\times 3600$ ). Fig. 60. Three-celled embryo ( $\times 1800$ ). Fig. 61. Pro-embryo (four-celled) ( $\times 2700$ ). Fig. 62. Pro-embryo (eight-celled) ( $\times 1800$ ). Fig. 63. Embryo with suspensor ( $\times 1260$ ). Fig. 64. Embryo with suspensor ( $\times 1800$ ). Fig. 65. Abortive embryo sac of *Ipomoea staphylina*.



In *Ipomoea Learii*, on the funicle slightly below the level of the micropyle, a group of cells enlarges and multiplies. As development proceeds, this tissue increases and fills up the gap between the carpellary wall and the micropylar region of the ovule. This nutritive tissue forms passage for the pollen tube at the time of fertilization. The existence of a tissue of such a function is interesting. The pollen tube makes its entrance down the style, which is of the common solid type.

#### FERTILIZATION

Fertilization takes place in the usual manner, the first male nucleus fusing with the egg, and the second with the polars. In the plants examined, the fusion of the polars is found to be delayed until the arrival of the second male nucleus. The two polars and the male nucleus fuse almost simultaneously in *Ipomoea Learii* and *Evolvulus alsinoides*.

#### ENDOSPERM

The fertilized egg undergoes a period of rest before dividing, while the primary endosperm nucleus divides almost immediately. The divisions are free-nuclear. In *Ipomoea Learii* the endosperm nuclei are found gathering around the growing embryo. In the advanced stages, when the embryo attains the full size, only traces of endosperm tissue are observed, most of it having been used up by the embryo. In *Argyrea speciosa* the fertilized egg seems to commence divisions without a period of rest. The first division of the fertilized egg occurs when the primary endosperm nucleus has undergone two or three free nuclear divisions. As the embryo proceeds in development, the endosperm nuclei gather at the micropylar portion of the embryo-sac leaving a space below. Wall formation takes place to form the endosperm tissue.

#### EMBRYOGENY

*Ipomoea Learii*, *Ipomoea hederacea*, and *Argyrea speciosa* are observed to follow the same type of embryogeny while *Evolvulus* radically departs from them in this aspect.

In the former group of plants, as a result of the first division in the transverse plane, the fertilized egg gives rise to a terminal cell towards the antipodal end, and a basal cell towards the micropyle (Figs. 34 and 46). The second division is radial and occurs only in the basal cell (Figs. 36 and 47). The terminal cell now divides by a transverse wall, so that the pro-embryo consists altogether of four cells, two at the base, divided by a vertical wall, and two at the tip separated by the transverse wall (Figs. 37 and 47). The penultimate cell again divides by a vertical wall (Figs. 37 and 47). The embryo thus consists of two tiers of two cells each, with a terminal cell towards the antipodal end. The terminal cell divides again by a transverse wall and the subterminal cell divides by a vertical wall, so that now the embryo consists of three tiers of two cells each and a terminal cell (Fig. 38).

This sequence of transverse divisions in the terminal cell and the vertical divisions in the resulting subterminal cell proceeds until the embryo is considerably elongated (Figs. 39, 48, 51 and 52). The basal group of cells undergoes divisions in all planes rapidly and enlarges (Figs. 40 and 53). After this stage, the sequence of further divisions cannot be traced with certainty. The terminal cell multiplies into a group of cells, which are small in size and rich in protoplasmic contents (Figs. 41, 42, 53, and 49). Difference in size and cytoplasmic contents differentiates these two groups of cells into the embryonal and suspensorial cells.

In *Ipomoea Learii*, the suspensor is many celled and pyramidal in shape, holding the embryo pendant at the broader end (Fig. 43). The cells at the broader end of the suspensor are larger in size, while those at the narrow end towards the micropyle region, are small and rich in cytoplasm. These cells enlarge further penetrating into the micropylar portion of the ovule eating up the tissues around (Fig. 44). Finally, the suspensor acquires an oblong shape. The continued activity of the suspensor cells is for the first time reported in the *Convolvulaceæ*.

In *Ipomoea hederacea* the suspensor is a globular mass of uniformly large cells (Fig. 49). The growth of the suspensor is definite in this plant unlike in the case of *Ipomoea Learii*.

In *Argyrea speciosa*, the suspensor is of no definite shape, but consists of many uniformly large cells (Fig. 54). The multiplying activity of the suspensorial mass of cells is short-lived, in this case also. In all these plants, the suspensor is haustorial in function.

The embryogeny of *Evolvulus alsinoides* differs radically from the three plants described. It conforms to the *Capsella*-type in the early divisions. The first division of the fertilized egg is transverse giving rise to the two-celled pro-embryo (Fig. 59). The terminal cell further undergoes transverse divisions, until the pro-embryo becomes a filament of seven cells (Figs. 60, 61 and 62). The terminal cell then undergoes a vertical division, giving rise to two cells (Fig. 62). These cells further divide anticlinally and periclinally to give rise to the embryo proper. The basal cell, which remains undivided till the formation of a vertical wall in the terminal cell, now divides by a radial wall. In other cells also of the filamentous suspensor radial divisions occur (Fig. 63). The hypophysis remains without undergoing a vertical division. After the first vertical division in the basal cell of the suspensor, several divisions follow in all planes to give rise to a globular base. Now the embryo assumes the shape of a dumb-bell (Fig. 64).

The cotyledons and the stem tip develop at the apical region, while the radicle develops at the base as usual. The origin of the stem tip is delayed in all the plants except in *Argyrea speciosa*.

#### DISCUSSION

The occurrence of sterility has been discovered in a number of plants and a number of views have been put forward to account

for this phenomenon. The factors that influence the fertility or sterility may be of a purely physiological and ecological, or cytological and morphological character. That the climatic factors induce sterility both in pollen and in embryo-sacs has been shown by a number of investigators.

Schürhoff<sup>23</sup> suggests that the pollen grains of *Eichhornia* become sterile due to inclement climates. Martin<sup>16</sup> observed ovular sterility and attributed it to moisture conditions. Stow<sup>26</sup> considered that the sterility of pollen grains in many varieties of potato was brought about by high temperatures. According to him, chromosomal aberrations supervene at higher temperatures, whereas at lower temperatures normal reduction division occurs in the pollen mother cells.

Chromosomal aberrations and cytological disturbances during meiosis are also other factors that bring about sterility. These aberrations may be lagging, non-disjunction of the chromosomes or disturbance of the genic balance, according to Darlington.<sup>7</sup>

Erlanson<sup>10</sup> finds that sterility in several Roses is due to structural hybridity and fragmentation.

In the species of *Ipomoea staphylina* studied, the present writer finds a high percentage of pollen grains to be morphologically sterile. This may be due to chromosome irregularities that occur frequently during the meiotic mitosis. These irregularities are deconjugation of the bivalents at diakinesis and metaphase of the heterotypic division and fragmentation of the chromosomes.

The recognition of the principle that the morphology of the pollen grain offers added support in tracing the phylogeny of Angiosperms, has been responsible for the publication of a number of papers on this aspect by several investigators. Wodehouse<sup>28</sup> thinks that spinescent, multi-pored pollen grains are more advanced than the round pollen grains with smooth exines and with few exit pores. On this basis the species of *Ipomoea* have a more advanced type of pollen grains than *Evolvulus*.

*Megasporogenesis.*—The general tendency among the Sympetalæ is towards the suppression of the parietal tissue, so that the primary archesporial cell functions directly as the megaspore mother cell. Exceptions have been reported with certainty, so far, only in the Cucurbitaceæ and the Plumbaginaceæ.

Peters (1909) reported the occurrence of parietal cell formation in *Cuscuta* and *Convolvulus*. This observation was seriously questioned by Dahlgren,<sup>8</sup> who attributed the reported occurrence to be due to the sections being cut tangentially.

Mathur<sup>16</sup> has recently (1935) reported the existence of parietal cells in *Convolvulus arvensis*.

The present investigation shows that in *Ipomoea Learii*, contrary to the course of development among Sympetalæ in general and the other species in particular, there is not only the formation of a

parietal cell, but the development of a parietal tissue as well. This is unique in Sympetalæ, and in this aspect seems to be a reversion to the condition prevalent among the Archichlamydeæ.

*Synergids*.—The formation of beak-like extensions in the synergids is a point of interest that has been reported in several plants. Such beak-like prolongations of the synergids are generally associated with long and narrow micropyles, and presumably help the passage of the pollen tubes.

Smith<sup>22</sup> (1898) reported beaked synergids in *Eichhornia* and Conard<sup>4</sup> found beak-like projections in the synergids of *Quercus*. Chamberlain (1897) found beaked synergids in *Salix* sp.

Among monocotyledons *Gymnadenia conopsea* develops beaked synergids according to Ward.<sup>27</sup> The majority of Compositæ investigated show a similar situation. Beaked synergids without a "filiform apparatus" have been observed in *Ipomoea Learii*, *Ipomoea hederacea* and *Argyrea speciosa* and in all probability serve to assist the progress of the pollen tube.

Another interesting feature which does not appear to have been reported before is the nucleolar fragmentation in the nuclei of the synergids. The nucleoli bud out a number of fragments, which extrude into the plasma substance of the synergids. The exact significance of this is not clear, but this extrusion may be a prelude to the general disintegration of the synergids.

*Embryogeny*.—The embryogeny of the Convolvulaceæ has been known but imperfectly. Hooker<sup>13</sup> reported the absence of cotyledonary development in *Cuscuta*. Macpherson<sup>15</sup> found polyembryony to be very common in *Convolvulus*. She observed that the fertilized egg divides by a transverse wall in the first division. The later development, according to her, appears to be the product of divisions in a number of planes and in no fixed order, resulting in embryos of irregular forms. Smith,<sup>21</sup> in a taxonomic and morphological study of a number of North Carolinian species, found the first division of the fertilized egg to be transverse in *Cuscuta arvensis*, the resulting cells being of unequal size, and the second being perpendicular to the first, occurring in the basal cell only. In *Cuscuta rostrata* he reports that the first division is transverse as in *Cuscuta arvensis*, the subsequent divisions occurring in both the cells of the pro-embryo.

The situation revealed by a study of *Ipomoea* species conforms to the course of development in *Cuscuta arvensis*, so far as the first two divisions of the pro-embryo are concerned. After this the trend of divisions differs almost radically and in no other case a similar kind of development has been reported before.

The nearest approach to a similar type of development appears to be in the embryos of the Leguminosæ, where the variation in the formation and differentiation of suspensor and embryo is very great according to Guignard.<sup>6</sup> The point of resemblance between the Leguminosæ and Convolvulaceæ is however, superficial, because

the developmental details within the two families are quite different. In the tendency of developing a massive suspensor, haustorial in function, the two are alike.

The sequence of divisions in the pro-embryo, as well as the origin and development of the multi-cellular suspensor in *Evolvulus alsinoides*, marks a total departure from the developmental details of the embryos, met with in the species of *Ipomoea* and *Argyrea*.

In *Evolvulus*, the first and the subsequent divisions of the fertilized egg are transverse and the pro-embryo is a filament of seven cells, unlike the type of *Argyrea* and *Ipomoea*.

Among the allied families, close resemblance can be traced to a limited extent between the embryo development of *Nolana prostrata* (Datta) and *Evolvulus alsinoides*. Datta<sup>9</sup> states that the first division of the fertilized egg is transverse and further transverse divisions give rise to a 6-celled filamentous pro-embryo. The embryogeny of *Evolvulus* parallels that of *Nolana prostrata* only upto this stage. Further development differs largely.

To nearly the same extent the embryo of *Evolvulus* parallels the embryogeny of *Myosotis hispida* investigated by Soueges.<sup>25</sup>

The multiplication of the basal cell into a globular suspensorial mass after the first vertical division in the terminal cell is an interesting feature. A near approach to the same condition is suggested in the embryo of *Asparagus* investigated by Robbins and Borthwick.<sup>18</sup>

## CONCLUSION

*Ipomoea Learii*, *Ipomoea hederacea*, *Ipomoea staphylina* and *Argyrea speciosa* are almost of the same size and habit as twiners. These closely agree in several morphological details also, and conform to the same type of embryogeny. Further, they have large spinescent pollen grains, with a hard exine and numerous exit pores.

*Evolvulus alsinoides*, which is a very small spreading herb, not only differs widely in its vegetative characters, but also shows a number of morphological differences in having quite a different type of embryogeny and producing infinitely small pollen grains with a smooth exine and one or two exit pores.

## SUMMARY

### 1. *Microsporogenesis*.—

- (1) The innermost parietal layer becomes the tapetum.
- (2) The mode of chromosome pairing is parasynaptic.
- (3) Diakinetic de-conjugation has been observed in both *Ipomoea Learii* and *Ipomoea staphylina* but is of more frequent occurrence in *Ipomoea staphylina*.
- (4) Sixteen bivalents are counted in the metaphasic plates of both *Ipomoea Learii* and *Ipomoea staphylina*.

(5) Perinuclear zone invests the heterotypic and homotypic spindles in *Ipomoea Learii*.

(6) The separation of the pollen grains is effected by furrowing.

(7) The exine forms a thick mesh-work at the angles of which spines develop in both *Ipomoea* species and in *Argyreia speciosa*.

(8) The pollen grains contain commonly two and rarely three nuclei at the time of shedding.

## 2. *Megasporogenesis*.—

(9) A single massive integument develops and rapidly overarches the small nucellus.

(10) In *Ipomoea Learii* the single hypodermal archesporial cell cuts off a primary parietal cell which divides further to form a parietal tissue and the megaspore mother cell.

In *Ipomoea staphylina* and *Evolvulus* the archesporial cell is hypodermal in origin and directly functions as the megaspore mother cell.

(11) The chalazal megaspore of the linear tetrad develops into the normal eight-nucleate embryo-sac.

(12) The fusion of the two polars is delayed until the second male nucleus approaches them.

(13) Syngamy is effected in the resting condition of the egg-nucleus and male nucleus.

(14) The fertilized egg takes a short period of rest in *Ipomoea Learii*, *Ipomoea hederacea*, and *Evolvulus alsinoides*, while it almost immediately commences to divide in *Argyreia speciosa*.

(15) The embryogeny of *Ipomoea Learii*, *Ipomoea hederacea*, and *Argyreia speciosa* is of one type, while the embryogeny of *Evolvulus alsinoides* radically differs from the above.

(16) The origin of the stem tip is slightly earlier in *Argyreia* than in the other plants described.

## ACKNOWLEDGMENTS

The writer feels extremely indebted to Dr. M. A. Samapathkumaran, M.A., Ph.D., for his kind guidance throughout the course of this work and valuable suggestions in the preparation of this thesis. He is also grateful to Mr. L. Narayana Rao, M.Sc., F.R.M.S., for his sympathetic help during the course of the above work. He feels particularly indebted to Mr. A. R. Gopala Iyengar, M.Sc., and the other members of the staff of the Department of Botany, Central College, Bangalore, for their very helpful criticisms throughout.

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## A METHOD FOR GERMINATING AND STAINING TELEUTOSPORES

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(Communicated by M. A. Sampathkumaran)

Received for publication on August 12, 1940

IN studying the life-history of rusts, a good knowledge concerning the germination and cytological details of the teleutospore is very essential. Successful germination of teleutospores has often been a difficult problem, as germination is determined by various factors like temperature, optimum moisture conditions, overwintering and others.

Plowright<sup>9</sup> germinated the teleutospores by floating them on water in hanging drops. Owing to excess of moisture and low oxygen pressure in these cultures, teleutospores develop long, promycelia, on which sterigmata develop into long slender branches, and basidiospores are produced only towards the surface of water. Similar phenomena have been reported in *Puccinia graminis* Pers., *Uromyces fabae* Pers., de Bary, *Phragmidium rubi-idaei* (DC) Karst by Blackman,<sup>1</sup> in *Puccinia Malvacearum* by Klebahn,<sup>7</sup> *Puccinia graminis* by Jaczewski.<sup>6</sup> Similarly Weimer reported<sup>10</sup> that teleutospores of *Gymnosporangium Juniperi Virginianae*, Schw. produced long promycelia if teleutospores were immersed in water. Occasionally in hanging drop cultures, in addition to abnormally long promycelia, the sterigmata instead of developing basidiospores become long filamentous threads.

Submerged spores do not germinate. If the moisture content is below the optimum requirement, germination is retarded after an initial development. Germination on water agar (15 gms. of agar, 1 l. of distilled water) has been successfully used by recent investigators. Dunegan<sup>5</sup> germinated over-wintered teleutospores of *Tranzschelia pruni-spinosa*, with the water agar method.

Spores germinated by means of these methods, cannot be fixed and stained easily. Blackman<sup>2</sup> working on *Gymnosporangium*, and Colley<sup>4</sup> on *Cronartium ribicola*, placed host tissue containing teleutospores in moist chambers, for germination, then fixed and sectioned the material after embedding in paraffin. This method cannot be adopted in cases where the host tissue is hard, forming cankers and tumours. Infection of the host tissue in moist chambers by saprophytic fungi inhibits spore germination.

The celloidin method suggested by Hans Kniep for staining mycelia was also adopted by the writer. Germinating spores are placed on a slide, and, as far as possible, water is removed by means

of an absorbent. One per cent. celloidin in ether alcohol is poured over the material, and the slide is allowed to dry. Celloidin forms a thin film over the material, and fixes it to the slide. In the process of clearing in clove oil after staining, celloidin dissolves in clove oil, and therefore clearing must be done quickly. When the slides are transferred to xylol, celloidin hardens again. In slides prepared by this method, nuclear details are not brought out distinctly, because of the layer of celloidin over the material.

A method followed by the writer for germinating and staining teleutospores of *Uromyces hobsoni* (Figs. 1 and 1a), and *Hapalo-*

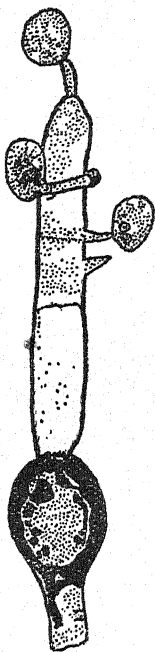


Fig. 1a. Camera lucida drawing of the same teleutospore showing binucleate basidiospores.  $\times 520$ .

*phragmium ponderosum* Syd. (found on *Acacia leucophlea*), (Figs. 2 and 2a), has given good results, and is as follows:—A drop of sterile distilled water is placed on a clean slide to which teleutospores scraped from the material are added. By gentle spreading of the drop with a glass rod, a thin smear is obtained on the slide. The slide is then allowed to dry for a few minutes, and inverted over a dish of water. The surface of water should not come in contact with the slide, and the two are separated by a large space.

After some time particles of water condense on the surface of the slide; thus affording conditions ideal for germination. When drops of water are placed on the back of the slide, the film of water

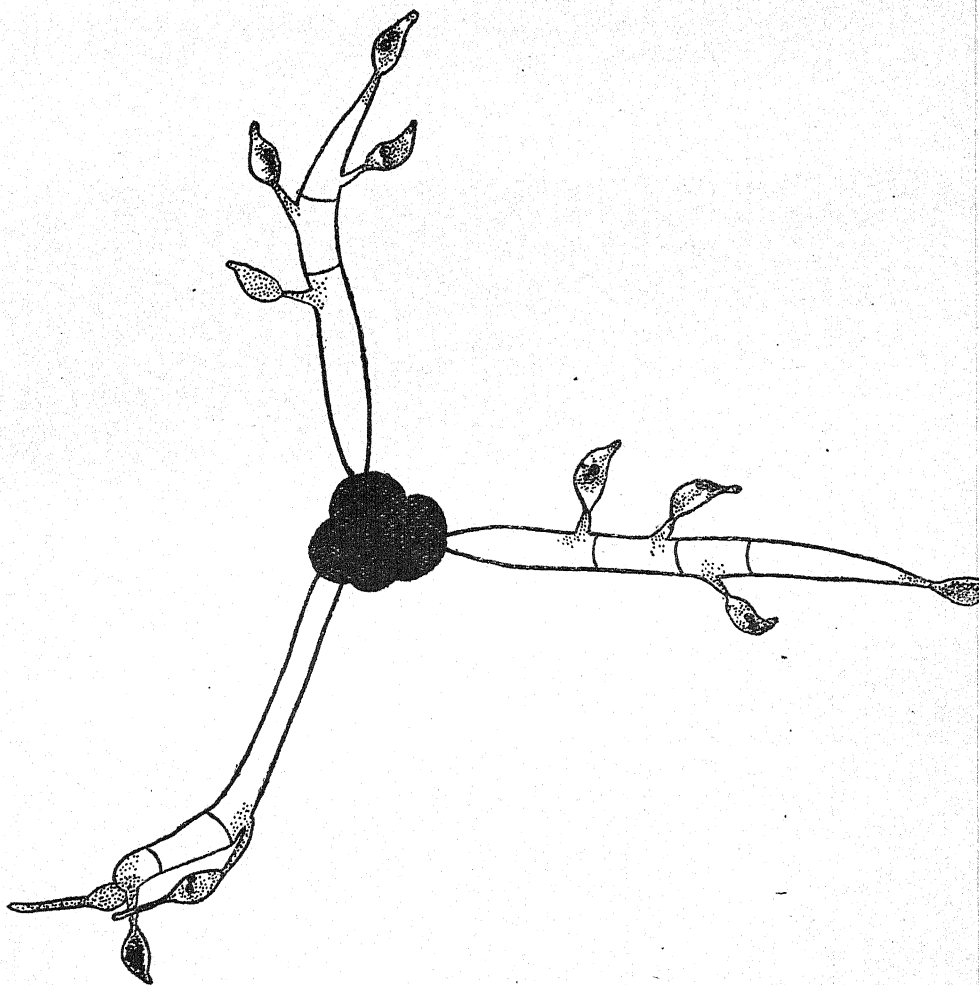


Fig. 2a. Camera lucida drawing showing the binucleate condition of basidiospores.  $\times 520$ .

is more readily condensed. The slides are frequently examined under the microscope and when large numbers of spores have germinated, the slides are transferred to a dish containing fixing solution. Ten per cent formalin water recommended by Chamberlain,<sup>3</sup> or Flemming's fluid gives good results. The slides are washed, stained in Heidenhain's iron-alum hæmatoxylin, with orange G or cosin in clove oil as counterstain, and finally sealed in balsam.

The advantage of this method is, that germination and staining of teleutospores is done on the slide itself. When the spores are mounted in sterile water and smeared on the slide, the spores get

firmly fixed to the slide, and do not get dislodged from the slide in the process of staining. This method is a modification of smear technique of pollen mother cells described by La Cour.<sup>8</sup> The spores are fixed to the slide before germination, and will not entail any drying after the spores have germinated. The fine particles of water condensed on the slide during germination, enclose large air spaces in between them. Consequently, excess of moisture and low oxygen pressure are avoided. This affords an optimum condition for germination. The slides are not overrun by contaminations from saprophytic fungi or bacteria. In the process of staining also the slides can be manipulated easily.

By following this method the writer was able to germinate and stain æidiospores and uredospores. The nuclear details are very conspicuously brought out (Figs. 3 and 3a). It is usual to germinate

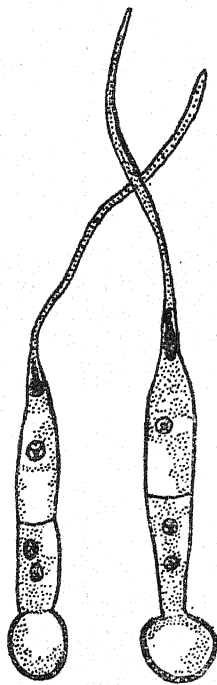


Fig. 3a. Camera lucida drawing of the germinating æidiospore.  $\times 520$ .

æidiospores and uredospores on hanging drop cultures on slides, and no satisfactory method has so far been suggested to fix and stain the germinating spores. Germination and staining of spores of other fungi can be attempted by following this simple technique.

In conclusion the writer wishes to acknowledge his indebtedness to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M., Professor of Botany,

Central College, Bangalore, for helpful suggestion and encouragement in the course of this work.

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## EXPLANATION OF PLATE I

- FIG. 1. Photomicrograph of germinating teleutospore of *Uromyces hobsoni* Vize., showing three basidiospores.  $\times 250$ .
- FIG. 2. Photomicrograph of triradiate teleutospore of *Hapalophragmium ponderosum* Syd., showing the germination of all the three teleutospores.  $\times 300$ .
- FIG. 3. Germination of æcidiospore of *Uromyces hobsoni* Vize., showing the two-celled germ tube, each having two nuclei. The migration of one of the two nuclei into the whip-like structure, can be clearly made out.  $\times 250$ .





SOME NEW PLANTS FROM INDIA  
AND BURMA

BY D. CHATTERJEE AND S. K. MUKERJEE

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(Communicated by K. Biswas)

Received for publication on July 20, 1940

1. *Uvaria merguiensis* Chatterjee, Sp. Nov. (ANONACEÆ)

PLANTA distincta; habitu ab *Uvaria macrophylla* Roxb. similis, sed ramis et foliis glaberrimis, floribus multo majoribus, pedicellis, longioribus bracteis angusto-lanceolaribus, sepala elongata, inter alia satis recedit.

A small tree, stem round, lenticellate rough, dark brown in colour, glabrous in all parts except in flowers. *Leaves* shortly petioled coriaceous, elliptic, obtuse entire, rounded at the base; midrib channelled throughout on the upper surface, prominently raised beneath, secondary nerves about 12 pairs distinct beneath; lamina 18 to 20 cm. long 9 cm. broad petiole stout, 7 to 8 mm. long. *Flowers* yellow 3 cm. in diameter; fascicled on small axillary tubercles, rarely solitary; pedicel 2.5 to 3.5 cm. long with one lanceolate bract in the middle. *Sepals* 3, slightly connate at the base, broadly deltoid, valvate, densely tomentose, 12 mm. long, 10 mm. broad. *Petals* 6, biseriate, outer petals larger much expanded and hooded above, densely tomentose on both sides, glabrous near the base on the inner side. *Stamens* many, connective produced, truncate, torus somewhat convex densely pubescent. *Carpels* many free, linear oblong pubescent, style very short thick with a dark glabrous neck and a hairy crown. Fruit not seen.

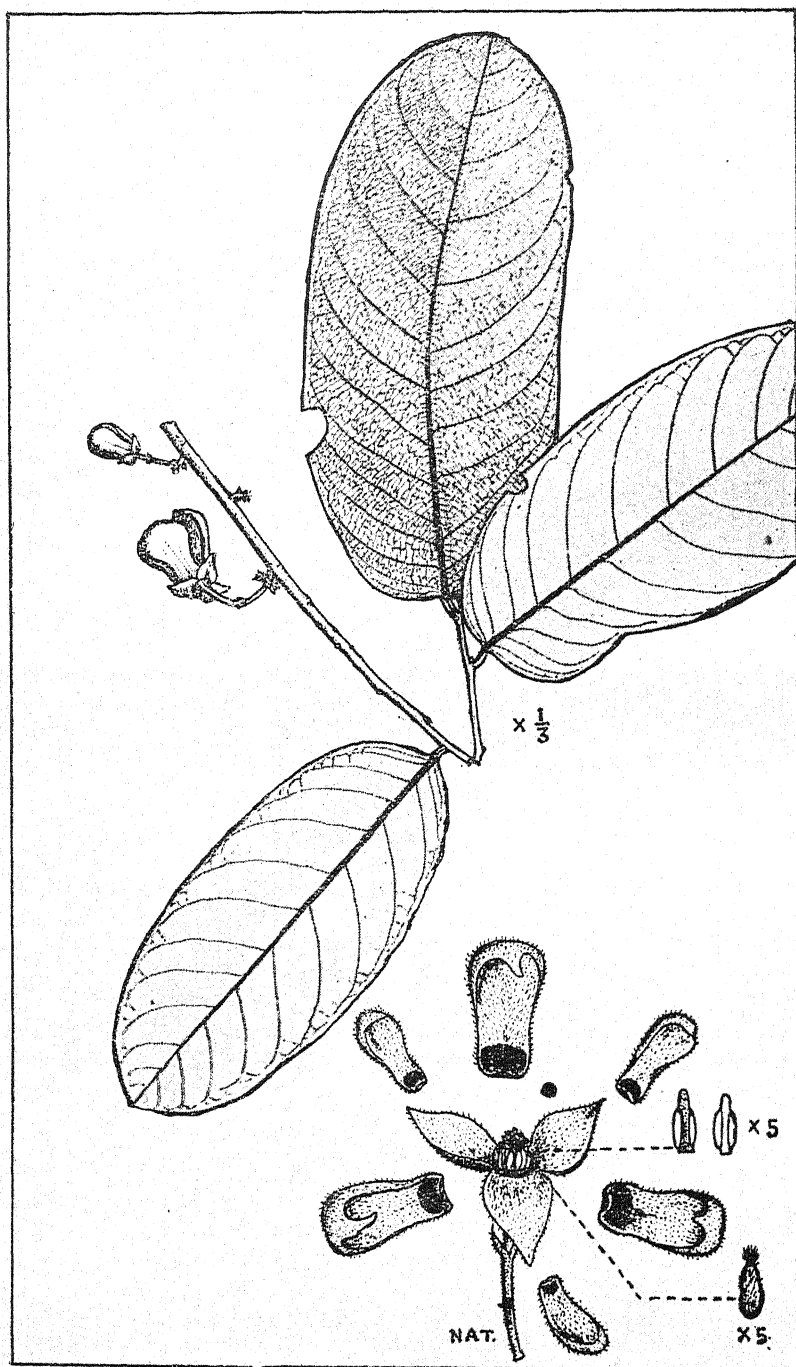
BURMA—Mergui, Victoria point *Po Khant* 11394 dated 28th March 1930 (Type in Herb. Calcutta).

The plant is distinct from almost all the Indian species of *Uvaria*. It resembles *Uvaria macrophylla* Roxb. in shape and size of leaves, but differs widely in its much bigger flowers, long pedicels, elongated sepals and narrowly lanceolate bracts.

2. *Goniothalamus tavoyensis* Chatterjee Sp. Nov. (ANONACEÆ)

*Goniothalamus subevenius* King similis, sed foliis majoribus, apices longi acuminatis, nervi approximantibus 14-20 paribus, floribus et pedicelli brevioribus bractae minoribus. sepala persistentibus, fructus majoribus, inter alia satis distinguitur.

A small tree; branches terete dark-brown in colour, lenticellate somewhat wrinkled, glabrous throughout. *Leaves* coriaceous shortly petioled, elliptic or narrowly elliptic, long acuminate,



Text-fig. 1. *Uvaria merguensis* Chatterjee Sp. Nov.—A flowering twig (reduced  $\frac{1}{3}$  nat. size) and dissection of flowers (natural). Stamens and carpel are shown magnified ( $\times 5$ ).

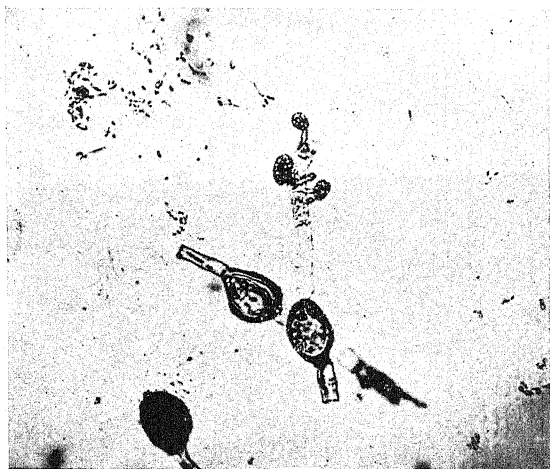


FIG. 1



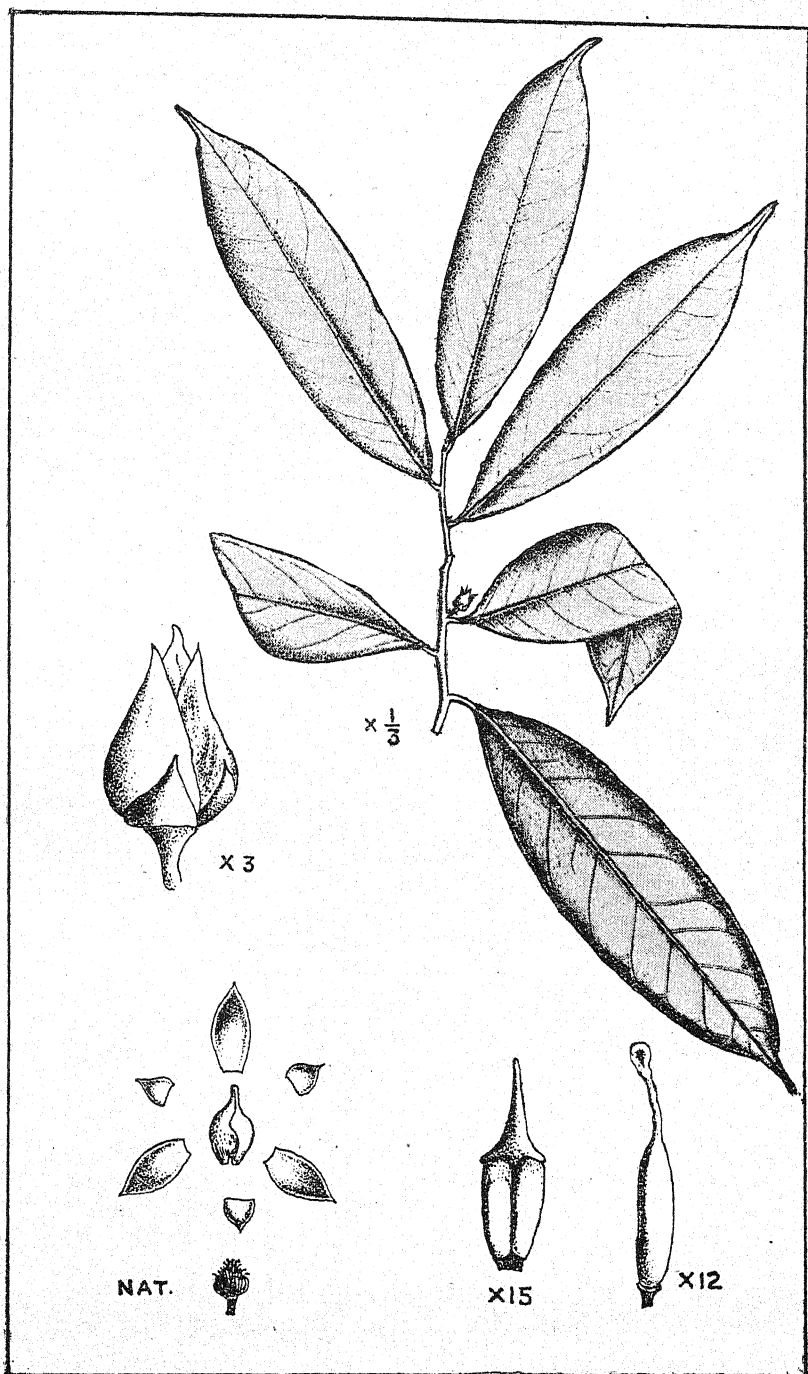
FIG. 3



FIG. 2

M. J. THIRUMALACHAR—A METHOD FOR GERMINATING AND  
STAINING TELEUTOSPORES





Text-fig. 2. *Goniothalamus tavoyensis* Chatterjee Sp. Nov.—A flowering twig (reduced  $\frac{1}{3}$ ) and dissection of flower (natural). Stamen and carpel magnified ( $\times 15$  and  $\times 12$  respectively).

margin entire revolute, base cuneate, glabrous on both surfaces, midrib raised below, channelled above throughout; main nerves 14 to 20 pairs anastomosing near the margin, lamina 10 to 24 cm. long, 3.5 to 6.5 cm. broad, petiole stout 5 to 10 mm long. *Flowers* small, solitary, axillary rarely supra-axillary, shortly pedicelled, bracts minute narrowly lanceolate, acute, pedicel 5 mm. long, stout. *Sepals* 3 free, valvate, deltoid ovate 4 mm. long glabrous, persistent in fruit. *Petals* 6, biseriate, thick, subequal; outer whorl free ovate lanceolate, acuminate, rusty, 8 mm. long, 4 mm. broad at the base; inner whorl cohering to form a cone-like vaulted cap over the stamens and ovary. *Stamens* numerous, free, very short, filaments absent, connective produced to a pointed process about as long as the anther; anthers lateral, elongate, 1.5 mm. in length with the produced connective. *Carpel* 7 to 8 free, narrowly cylindrical glabrous, style elongated stigma spatulate; *ripe carpels* shortly stalked 4 to 8, ellipsoidal, acutely hard pointed, 14 to 18 mm. long, 8 to 10 mm. broad, glabrous; main stalk stout 9 to 15 mm. long, secondary stalk 2 to 3 mm. long. *Seeds* solitary, white or light-yellow, shining 12 to 13 mm. long, slightly compressed.

BURMA—Tavoy, Heinze Camp 550 m. *P. T. Russel* 2025 (Type in Herb. Calc.), 2187 & 2199.

This species was left undescribed among the Burmese sheets in the Herbarium. It resembles to a certain extent with *G. subvenius* King and *G. tapis* Miq. in the shape of its leaves, but differs distinctly in long acuminate, apices, smaller flowers, short pedicles, persistent sepals and larger ellipsoid fruit.

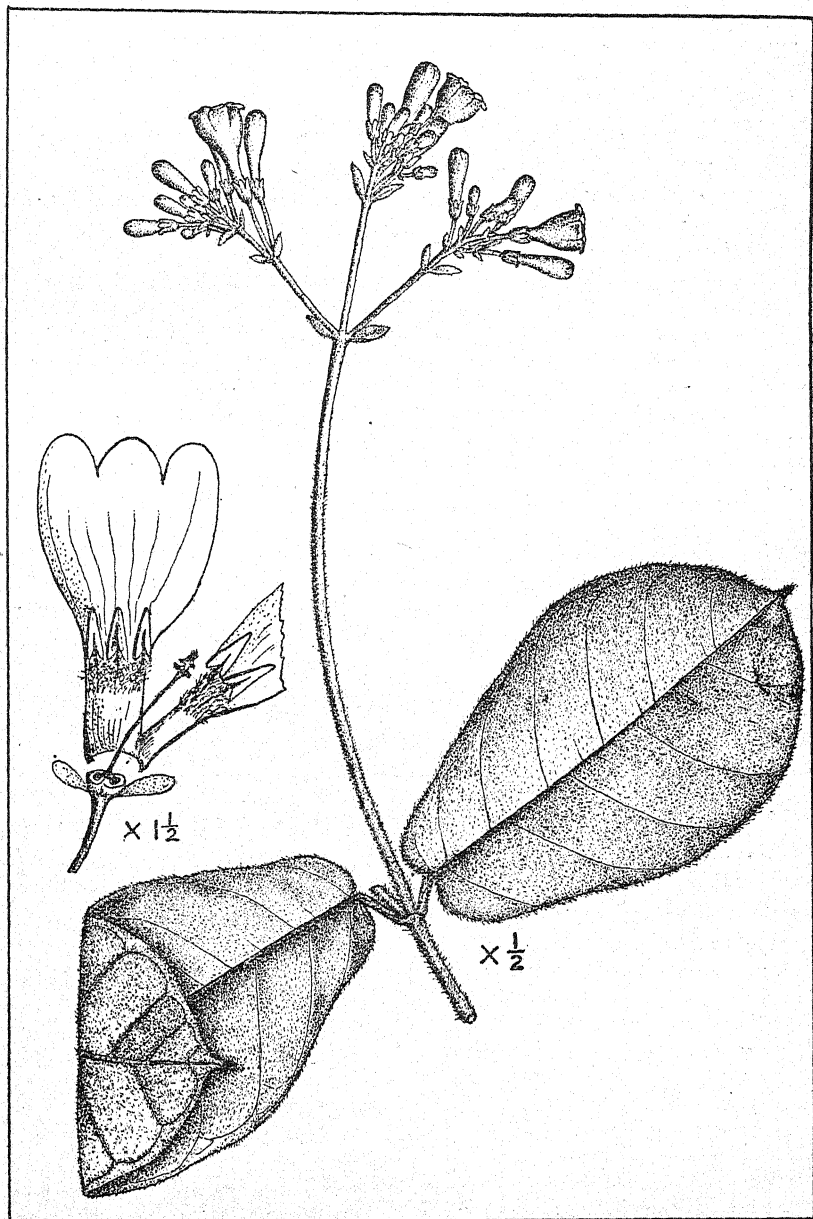
### 3. *Amalocalyx burmanicus* Chatterjee Sp. Nov. (APOCYNACEÆ)

Affinis *Amalocalyx microlobus* Pierre sed, foliis majoribus, obovatis, apice acuminatis, basi cordatis, inflorescentis longe pedunculatis, floribus majoribus, inter alia satis recedit.

A climbing herb, growing generally on hedges; all parts densely and softly tomentose with minute brown hairs, internodes rather long. Stem terete, ribbed, soft and fistular. *Leaves* shortly petioled, opposite, broadly obovate, cordate at the base, margin entire, apex cuspidately acuminate midrib grooved above, slightly raised below, main nerves 8 to 11 pairs anastomosing near the margin distinct below; lamina soft and somewhat membranous, lower surface of the leaf always densely tomentose, specially so on the nerves; upper surface less hairy in older leaves; lamina 11 to 20 cm. long, 5 to 14 cm. broad; petiole 1.5 to 3 cm. long channelled above. Inflorescence; usually trichotomously branched cymes occurring in terminal or axillary branches, forming three groups at the end, (of 8 to 10, flowers in each group); bracts and bracteoles present, caducous, membranous, bracts, .5 to 1 cm. long, broadly ovate; main peduncle 10 to 15 cm. long secondary peduncles 2.5 to 4 cm. long. *Flowers* regular bisexual dark red, hoary outside, gamopetalous, pedicels slender 1 to 2.2 cm. long. *Calyx* tubular, campanulate, connate at the base, sepals 5, lanceolate with obtuse apex, membranous



tomentose, 4 to 7 mm. long. *Corolla* gamopetalus, funnel-shaped, tube narrow and slender below much dilated above, lobes 5, short imbricate, recurved outwards, pubescent outside with minute hairs on the limb and with a ring of hairs inside near the throat. *Stamens*



5 included, epipetalous, filaments very short densely hairy, anthers appendiculate equal, sagittate, 4 mm. long with long short filament. *Carpels* of 2 ovaries distinct, style about 12 mm. long., stigma cylindrical 2 mm. long, constricted at the middle, apex conical and densely hairy, apices of anthers and stigma at the same level. *Fruit and seed* not seen.

BURMA—Shan Hills natik pap 1300 m. *Collett* 734 (Type in Herb. Calcutta); Upper Burma, *Prazer* without number; Southern Shan States, Kengtung 1000 m. *Mc. Gregor* 555.

Zibingyi near Maymyo Plateau 650 m. *J. H. Lace* 6221.

YUNNAN—"Henry 9565 at Kew Herb. agrees well with *Collet* 734 and probably represented this species"—W. G. Craib, Kew.

The discovery of this plant from Burma is interesting as a new record of the Indo-Chinese genus *Amalocalyx* as well as an addition to the hitherto monotypic genus. The four sheets in Herb. Calcutta are all in flowers and were first regarded as some species *Chonemorpha*. It was sent to Kew in 1913 with the remark, "A small flowered *Chonemorpha* Sp. Is it at Kew under any name?" Late Professor W. G. Craib who compared these sheets at Kew, wrote back while returning the sheets as follows:—

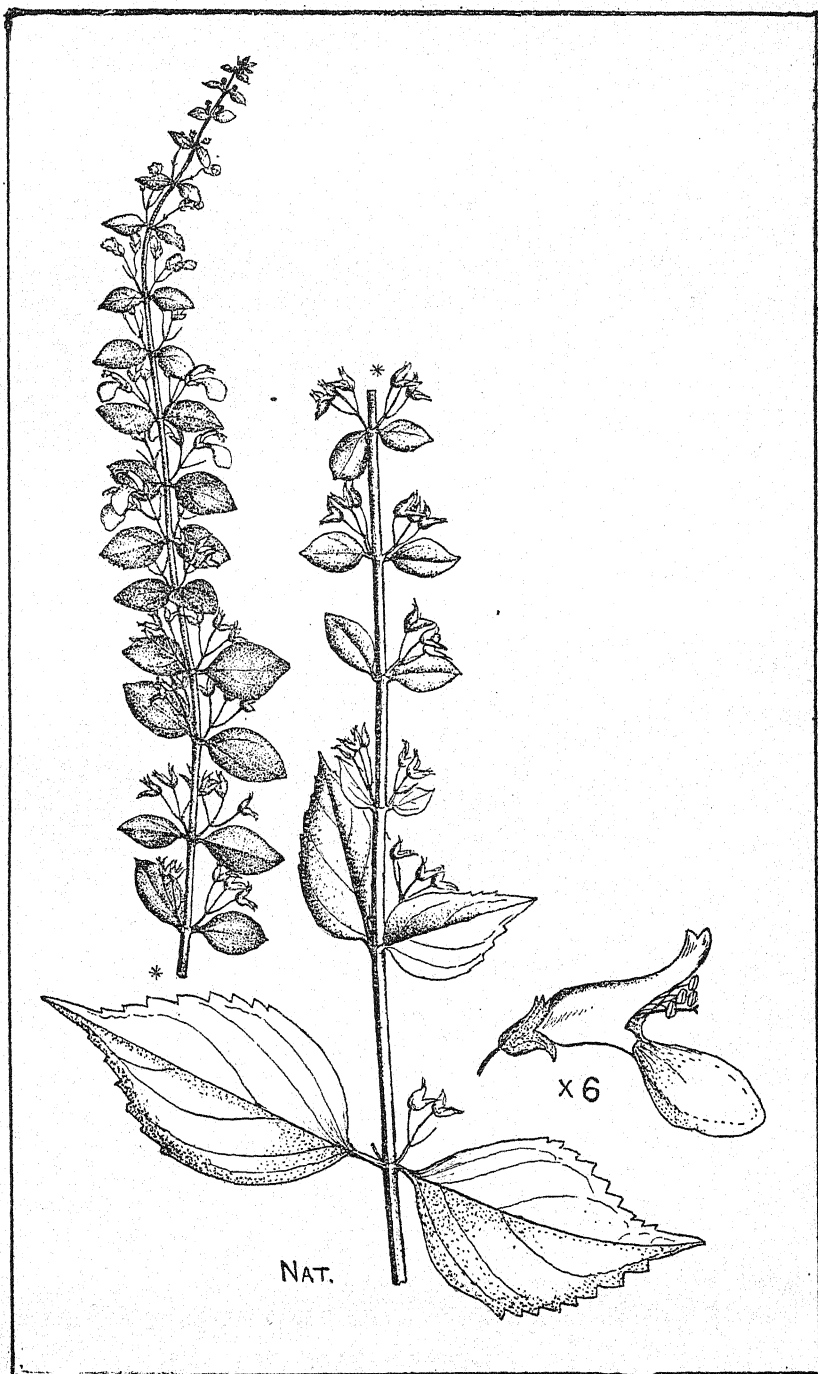
"This I believe *Amalocalyx microlobus* Pierre, but we have no type of that species but we have Pierre's drawing which agree fairly with yours." "Since writing this note I have seen the type of *A. microlobus* Pierre and now regard *Collett* 734 = *McGregor* 555 as a new species of the genus"—(Sd.) W. G. Craib, December 20, 13.

I have compared the description and drawing of *Amalocalyx microlobus* Pierre [*Lecomte Flor. Gen. Indo China*, Part 3 (1922-23)] and consider that Craib was right. This species is very much allied to the above but differs in larger leaves, longer peduncles, trichotomously branched cymes and much inflated corolla.

#### 4. *Plectranthus assamicus* Mukerjee, Sp. Nov. (LABIATÆ)

*P. mellisoides* Benth. similis, sed foliis majoribus, regulariter, acute serratis, acuminatis, ad basi late rotundatis bracteis conspicuis, inter alia differt.

Erect herb, stem quadrangular, grooved, minutely adpressedly hairy. *Leaves* (lower not seen) opposite, petioled, broadly ovate, acuminate, serrate up to the middle from above, entire near the rounded base, sparsely hairy, glandular punctate; lamina 4-4.5 cm. long, 2-2.5 cm. broad; petiole slender, 3-5 mm. long. *Cymes* distant, on a lax-flowered, long, slender, erect raceme; lower cymes axillary; bracts foliaceous, shortly petioled, ovate, entire, 5-12 mm. long, 4-8 mm. broad; pedicles slender as long as or longer than the fruiting calyx. *Calyx* campanulate, 2 mm. long, puberulous, 2-lipped, upper lip 3-toothed, teeth acute, slightly recurved, lower lip longer than the upper, with 2 narrow acute teeth; fruiting calyx



Text-fig. 3. *Plectranthus assamicus* Mukerjee Sp. Nov.—A flowering twig (natural) and a flower magnified ( $\times 6$ ).

4-5 mm. long, 3-4 mm. wide. *Corolla* 7-8 mm. long, tube short, broad, gibbous at base; lower lip 4 mm. long, upper shorter. *Stamens* glabrous, upper pair longer, exserted. *Style* included. *Nutlets* globose, compressed, 1.5 mm. diam., deep brown, minutely punctate or smooth.

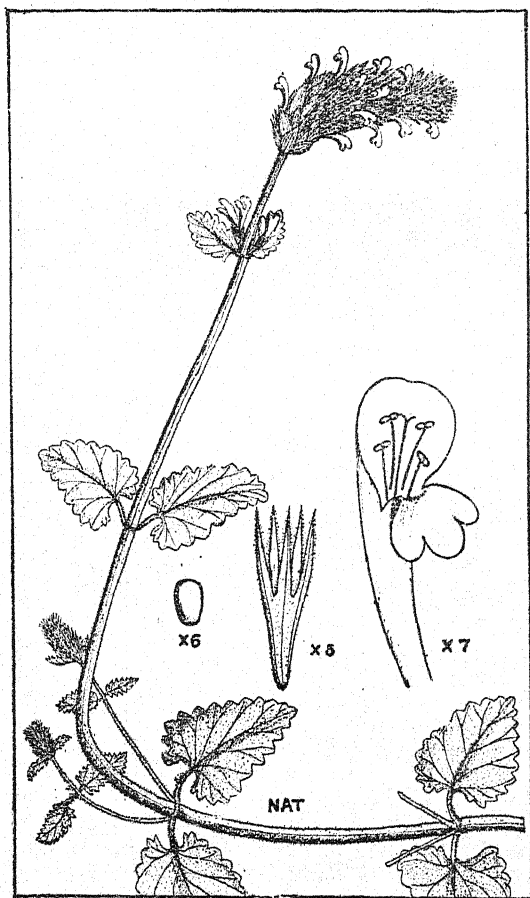
ASSAM—Akha hill, *Bor* 1364. (Type in Herb. Cal.).

The plant is quite distinct from the other species of *Plectranthus*. It bears some similarity to *P. mellisoides* Benth. which is also confined to the hills of Assam. The two species resemble in the nature of their inflorescences and calyx but differ from each other in the shape and size of leaves and bracts.

5. *Nepta Duthiei* Prain et Mukerjee Sp. Nov.

(LABIATÆ)

Affinis *Nepeta spicata* Benth., sed folia cordata, apice rotundata pagina inferiore floccosa, petiola valde brevipes, inter alia differet.



Text-fig. 4. *Nepeta Duthiei* Prain et Mukerjee Sp. Nov.—A portion of the plant with inflorescence (natural). Parts of flower shown separately.

Stout erect herb, upto 35 cm. high. Stem more or less rounded puberulous. *Leaves* petioled, slightly thick, broadly ovate cordate at the base obtuse margin crenate; nerves prominent; upper surface densely and minutely tomentose slightly rugose, lower surface floccose and white; lamina 1.5 to 3 cm. long., 1 to 2.5 cm. broad; petiole about 1 cm. long below smaller above rusty villous. *Spike* dense, terminal often with an isolated whorl below, about 3.5 cm. long densely hairy with soft and glistening hairs; bracts membranous, ovate, apiculate about 5 to 7 mm. long 3 to 5 mm. broad. *Calyx* 7 mm. long teeth narrow subulate plumose, larger, almost equalling the calyx tube. *Corolla* about 12 mm. long tube exerted. *Stamen* included. *Nutlets* 1.5 mm. long obvate smooth brown in colour.

WEST HIMALAYA—Tihri-Garhwal Ruddee Ghore ka Gadh 10-11000 ft., J. F. Duthie—171, d. July 19, 83. (Type in Herb. Dehra Dun, co-type in Herb. Cal.) Kumaun, Ralam valley, 12-13000 ft., J. F. Duthie 3304, d. August 23, 84.

This species resembles *Nepeta spicata* Benth. from which it is easily distinguished by the cordate leaves with floccose undersurface. It was previously named as *Nepeta floccosa* Benth. probably for the floccose nature of its leaves. but could be easily separated by its distinctly different type of inflorescence.

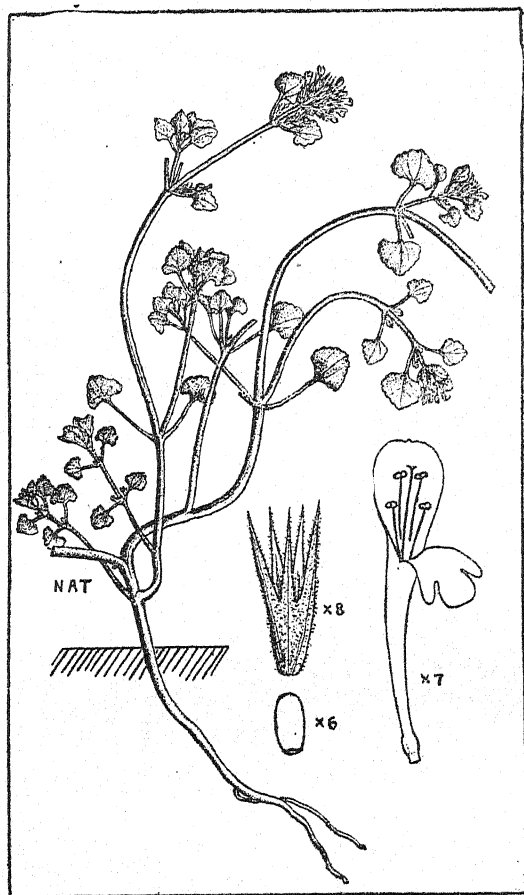
6. *Nepeta Gilesii* Mukerjee Sp. Nov. (LABIATÆ)

Affinis *N. floccosa* Benth., sed ramis folisque minoribus laxè pubescentibus, florum verticillis axillaribus differet.

Small fleshy herbs, erect or ascending, 7-15 cm. high, branching or not, floccosely woolly at younger parts. *Leaves* petioled, thick rhomboid or deltoid-ovate, rounded at apex, truncate or slightly cuneate at base, margin crenate or sinuate or subentire, laxly hairy with long white hairs on both surfaces specially when young, and densely so on the petiole; lamina 5-15 mm. in diam., petiole 5-15 mm. long; nerves faint. *Flowers* few in axillary whorls at the ends of branches, whorls shortly peduncled; bracts petioled, ovate or often linear, apiculate, cuneate at base, entire. *Calyx* glandular, 5 mm. long, slightly enlarged in fruit, curved and with an oblique mouth, when young covered with a floccose wool disappearing afterwards; teeth more than half the length of the tube, linear-subulate. *Corolla* 9 mm. long, pubescent; tube slender dilated abruptly at base, limb short. *Stamens* included, upper pair inserted at the middle of the tube, lower pair a little below the mouth. *Nutlets* 2 mm. long, elliptic, compressed, brown in colour.

WEST HIMALAYA—Gilgit Expedition (N. of Hindukush), Coll. Dr. Giles, "Recd. through Mr. Duthie, Jan. 1887". (Type in Herb. Cal., co-type in Herb. Dehra Dun.)

This is a distinct species of the genus resembling *N. floccosa* Benth. by being floccosely hairy and also by the thick leaves. The two species can be readily distinguished by the type of inflorescence and also by the size of leaves etc.



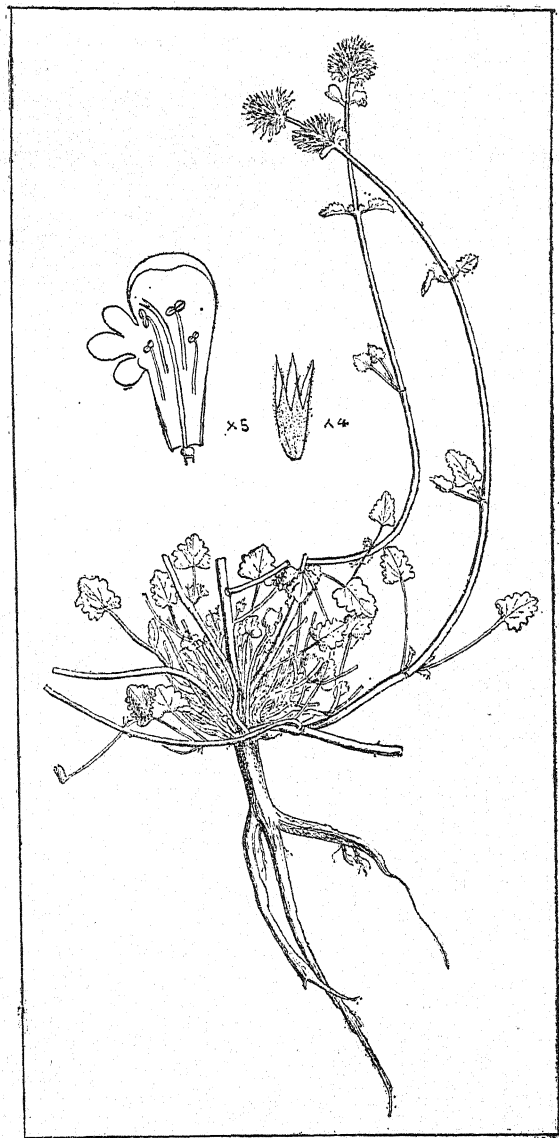
Text-fig. 5. *Nepeta Gilesii* Mukerjee Sp. Nov.—A part of the plant (natural) and a dissected flower magnified ( $\times 7$ ), calyx ( $\times 8$ ) and nutlet ( $\times 6$ ).

7. *Nepeta multicaulis* Mukerjee Sp. Nov. (LABIATÆ)

Ab affinis habitu *Nepeta bracteata* Benth., sed foliis basi cordatis, apice rotundatis, bracteis lanciolatibus et valde brevioribus distinguiter.

Small tufted herb, about 15 cm. high branches many from the base, ascending, slender, 4-angled, dark pinkish in colour, minutely puberulous. *Leaves* distant, lower long petioled, slightly thick, ovate, obtuse, cordate at the base, lobed at the margin, glabrous above, sparsely hairy below; nerves sunk on the upper surface, glands very prominent below; lamina 5 to 9 mm. long, 4 to 8 mm. broad; petiole slender, puberulous, about 4 cm. long at the base upper smaller, almost none near the spikes. *Flowers* pedicelled, in

short compact villous spikes terminating each branch, usually minute, 1-2 mm. long. *Calyx* 5 mm. long villous, calyx teeth aristate, smaller than the tube. *Corolla* 8 mm. long; tube as long as calyx. *Stamens* included. *Nutlets* not seen.



Text-fig. 6. *Nepeta multicaulis* Mukerjee Sp. Nov.—Plant with flowering shoot; dissections of calyx and corolla are shown magnified.



N.W. INDIA—Baltistan-Saltaro valley, little Thibet, No. 10266 A. G. Hunter-Weston, R.E., d. May 28, 90. (Type in Herb. Dehra Dun).

This species is allied to *N. bracteata* Benth. in the general habit and the nature of inflorescence. It differs from *N. bracteata* in having its leaves cordate at the base and rounded at the apex; bracts much smaller and lanceolate.

8. *Nepeta paucifolia* Mukerjee Sp. Nov. (LABIATÆ)

Plansta distincta. Flores et inflorescentis *Nepeta connata* Royle ex Bth. et *Nepeta linearis* Royle ex Bth. similis, sed foliis brevioris ovatis obtuso serratis (specimina *N. connata* Royle at *N. linearis* Royle foliis angustioribus linearis vel lineari-lanceolatis) interalia differet.

Erect slender herb, about 30 cm. high, stem obtusely 4-angled, minutely puberulous; branches few. *Leaves* shortly petioled or almost sessile, membranous, broadly ovate, smaller leaves often obovate, obtuse, cuneate or rounded at the base, margin serrate, glabrous on both surface, with few scattered hairs on the nerves beneath; lamina 2.5 to 3 cm. long 1 to 2.5 cm. broad, petiole 2 mm. long or shorter. *Spikes* short dense terminal; bracts 2 at the base of the spike, linear lanceolate, acute and stipulate at the apex, entire or shortly toothed at the margin, 8 to 10 mm. long; bracteoles many filiform plumosely hairy. *Calyx* 9 mm. long densely pubescent with glistening hairs, calyx tube slightly curved outwards, teeth unequal, subulate largest 3 mm. long. *Corolla* 12 to 15 mm. long tube slender slightly longer than the calyx, limb expanded. *Nutlets* 1.5 mm. long obovate truncate at the top smooth dark brown in colour.

N.W. INDIA—Baltistan, Karpucha nublut (?) 11-12,000 ft. J. F. Duthie July, 9, 92. (Type in Herb. Dehra Dun.)

The plant is different from the other species of *Nepeta*. It approaches *Nepeta connata* Royle ex Bth. and *Nepeta linearis* Royle, ex Bth., as regards the character of the inflorescence and flowers, but differs from both in the character of the leaves. The leaves are ovate and broadly serrated, in comparison with much elongated linear or linear-lanceolate leaves of *N. connata* and *N. linearis*.



Text-fig. 7. *Nepeta paucifolia* Mukerjee Sp. Nov.—A flowering branch with dissection of a flower. Calyx, corolla and nutlet shown magnified.



## STUDIES ON THE PHOTOCHEMICAL ACTION IN PLANTS

### II. Photosynthesis in leaves at different temperatures

BY SHRI RANJAN

Received for publication on August 30, 1940

#### INTRODUCTION

THE study of photosynthesis as related to temperature is an old theme and it would seem a little too daring to attempt a new interpretation of such a relationship. But in view of certain observations on the effect of light on respiration made by the author the whole idea of photosynthetic rate has to be reconsidered. It is superfluous to refer to the vast amount of work already done on the subject and those interested in the historical part are referred to well-known text-books on the subject.

#### MATERIALS AND METHODS

The experiments were conducted on excised leaves of *Eugenia jambolana*, which were placed in a plant chamber made of brass with inlet and outlet tubes, having one of the surfaces made of glass which could be fitted on to a groove and sealed with wax. The dimensions of the plant chamber were 22.5 cm. by 10 cm. Most of the inner space of the plant chamber was filled with wax in order to reduce the amount of space in it. The leaves were placed in the plant chamber, the petiole dipping in a few drops of water and then the plant chamber placed in a Hearson's cool incubator maintained at a constant temperature either by ice and electricity, if it had to be kept below room temperature or, at higher than room temperature, by electricity alone.

A current of  $\text{CO}_2$  free air was passed through the plant chamber which then bubbled through a standard solution of  $\text{Ba}(\text{OH})_2$  placed in Pettenkofer tubes. The current of air was switched on from one Pettenkofer tube to another every 2 hours by means of a clock-work and Blackman's air current commutator. After a few hours of respiration, the plant chamber was exposed to light, the source of which was a 1500 watt Osram Bulb placed at a distance of 1 ft. Light was passed through a rectangular jar 4" wide through which a current of cold water was circulated in order to check heat radiations reaching the plant. During the period in light the plant chamber was supplied with 0.2%  $\text{CO}_2$  instead of  $\text{CO}_2$  free air.  $\text{CO}_2$  in bulk was prepared by the action of  $\text{HCl}$  on marble and stored in a gas cylinder. Requisite quantity of this gas was mixed with air before entering the plant chamber. Part of the air so mixed with  $\text{CO}_2$  was also led directly by means of a Y-tube

to another set of Pettenkofer tubes and the  $\text{CO}_2$  content of the current estimated. After the period in light the leaves were again allowed to respire in dark in  $\text{CO}_2$  free air.

#### GAS REGULATORS

In order that the flow of gas through the experimental chamber and through the set of Pettenkofer tubes directly concerned, during the period in light, be exactly equal the following device was used which proved highly satisfactory. A platform balance was used. On each pan of the balance was placed a water container "A" Fig. 1 of sufficient capacity. The bottom of the container pressed

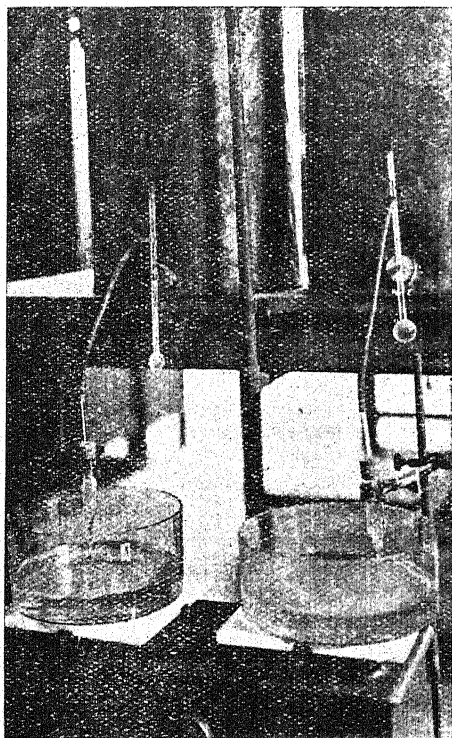


FIG. 1

against the glass rod "C" of the gas regulator, the upper end of which fitted into the lower end of the tube "B" 3" long by 1" in diameter. The contact surfaces of B and C were ground leaving a very minute groove so that water could very slowly flow out when fully closed, Fig. 2. The upper end of B was connected with an outlet of an aspirator. From the aspirators, water passed out of "B" and collected in "A". Any unequal collection of water in "A" disturbed the balance so that the platform on one side was

raised up. This caused "C" to be pressed into "B" and consequently the ground surfaces being separated a greater flow of water out of "B" took place. As this happened the amount of water in

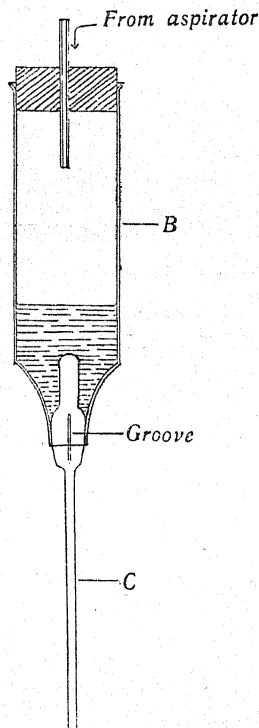


FIG. 2

"A" was increased causing the platform of the balance to go down, so that "C" came to its original position and the outflow of water decreased. While on the other platform of the balance the reverse of this took place and the flow of water increased. By this device the amount of outflow of water from the aspirators could be kept fairly equal. By measurement it was found that differences not amounting to more than  $\pm 1$  c.c. per hour occurred in the amounts of water collected on each side.

The respiration and assimilation rates were estimated every two hours at 20° C., 25° C., 30° C., and 35° C.

#### DISCUSSION OF THE RESULTS

Experiment No. 1 which is represented by Fig. 3 was carried out at 20° C. The respiration in both the control and experimental set followed a level course. Light and 0.2% CO<sub>2</sub> was given after 22 hours and the photosynthetic rate (*i.e.*, the CO<sub>2</sub> taken in by the leaves) marked A in the Fig. showed a fairly constant level. When

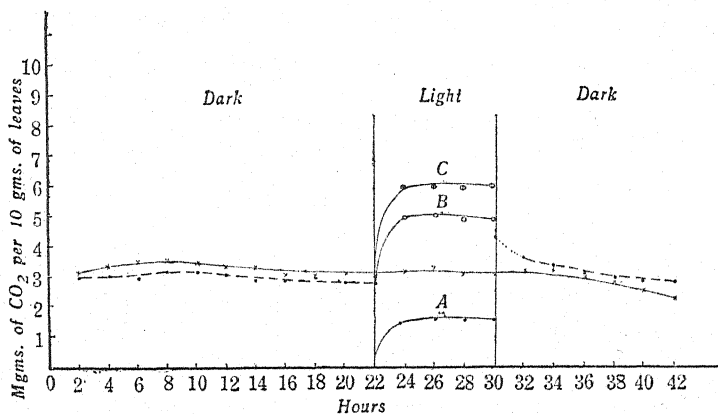


FIG. 3

light and  $\text{CO}_2$  were removed, a slight after-effect on the respiration rate was noticed.  $\text{CO}_2$  emission started higher than the control and gradually fell off. The control set which was always in dark showed a slightly falling curve throughout.

In experiments 2, 3 and 4 (Figs. 4, 5 and 6) the general plan of experimentation was the same as in experiment 1 except that the temperatures were kept at  $25^\circ$ ,  $30^\circ$  and  $35^\circ$  C, respectively. The general response of the plant in light and darkness was also very much the same.

The trend of respiration curves at different temperatures and the effect of light on respiration has been discussed in a previous paper of this series.<sup>1</sup> We are now concerned with the photosynthetic rate and the effect of temperature on actual assimilation.

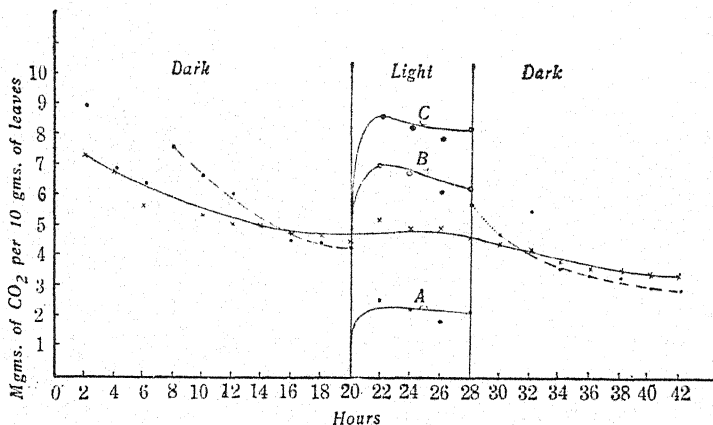


FIG. 4

<sup>1</sup> "Studies on the photochemical action in plants. (1) The respiration of entire *Pistia* plants in light," *Jour. Ind. Bot.*, 1940.

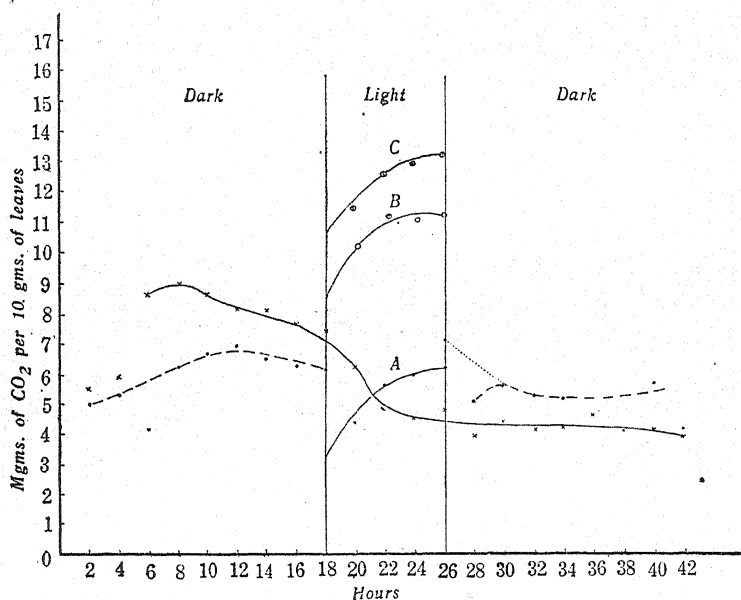


FIG. 5

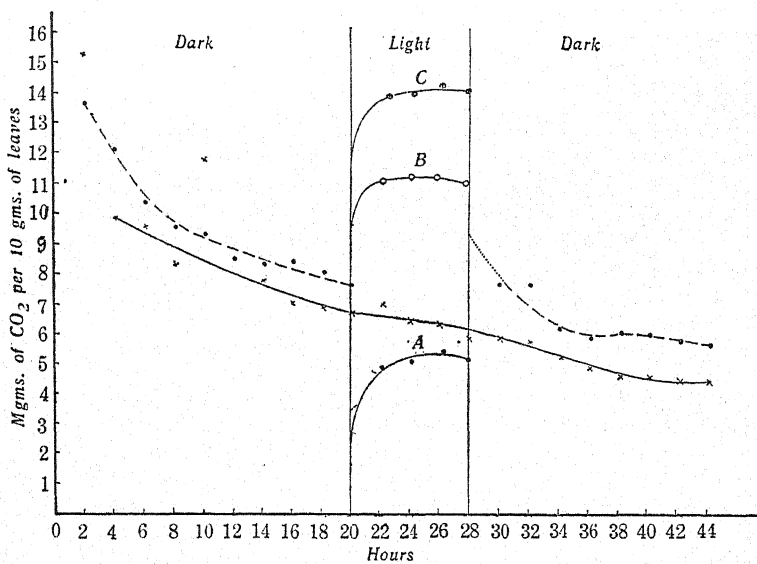


FIG. 6

INTAKE OF CO<sub>2</sub> AT DIFFERENT TEMPERATURES

In all cases 0.2% CO<sub>2</sub> was supplied to the plants when illuminated. At 20° C the photosynthetic rate as measured by intake



of  $\text{CO}_2$  (marked A in Fig.) was 1.6 mgm.  $\text{CO}_2$  per 20 gm. hour of leaves, which kept constant throughout the time assimilation rate was measured. At  $25^\circ \text{C}$  this rate, however, increased to 2.7 mgm.  $\text{CO}_2$  and then showed a slight decline to 2.3 mgm. At  $30^\circ \text{C}$  the rate further increased to 4.5 mgm.  $\text{CO}_2$  which increased with time. And at  $35^\circ \text{C}$  the  $\text{CO}_2$  intake rate was highest being about 5.0 mgm.  $\text{CO}_2$  which kept fairly constant. Thus with increase of temperature the rate of photosynthesis gradually increased. Supposing the rate of photosynthesis at  $20^\circ \text{C}$  be unity, the same at  $25^\circ \text{C}$ ,  $30^\circ \text{C}$  and  $35^\circ \text{C}$  would be 1.7, 2.8 and 3.1 respectively.

#### THE ACTUAL ASSIMILATION RATE

However, the rate of intake of  $\text{CO}_2$  is not the actual rate of photosynthesis since the amount of  $\text{CO}_2$  given out in respiration during the same period is also involved and used up in the photosynthetic process. Hence in order to get at the actual rate of assimilation the respiratory  $\text{CO}_2$  output should be added on to the  $\text{CO}_2$  intake rate as recorded. The actual assimilation rate as shown in the graphs (marked B) has been obtained from the  $\text{CO}_2$  intake in light and the respiratory rate during the same period as measured in the control set of leaves in dark. Thus the actual photosynthetic rate at  $20^\circ \text{C}$ ,  $25^\circ \text{C}$ ,  $30^\circ \text{C}$ , and at  $35^\circ \text{C}$  comes to the mean values 4.8, 7.3, 10.7 and 11.2 mgms.  $\text{CO}_2$  and taking the rate at  $20^\circ \text{C}$  as a unity the other rates work out to be 1.5, 2.2 and 2.3 respectively.

#### DERIVED REAL PHOTOSYNTHETIC VALUE

In the previous paper of this series it was shown that the respiratory rate in light also increases over the rate in dark. It was discussed in the same paper that in order to derive this respiration rate in light, the subsequent respiration curve in dark is to be produced backwards to the end of the period of illumination *i.e.*, the zero hour of darkness after light. The dotted line in each graph represents such drift of respiration interpolated backwards. This value, therefore, gives the measure of respiratory rate in light and in order that the real photosynthetic rate be derived this has to be taken into consideration. The curve in light in each graph represented by Figs. 3-6 (marked C) represents this derived real photosynthetic value, which stands, at 6.0, 8, 13 and 14 mgm. at  $20^\circ \text{C}$ ,  $25^\circ \text{C}$ ,  $30^\circ \text{C}$ , and  $35^\circ \text{C}$  respectively. Again taking the value at  $20^\circ \text{C}$  to be unity the same at other temperatures are 1.3, 2.1 and 2.3 respectively.

Thus the derived real photosynthetic value is very much greater than the actual intake of  $\text{CO}_2$  and greater than the so-called real assimilation rate. In Fig. 7 this real assimilation rate (marked B) and the derived real photosynthetic values (marked A) at different temperatures are shown. The curve B shows a steady rise with increasing temperature upto  $30^\circ \text{C}$ , beyond this temperature the rise however falls off and the curve tends to assume a horizontal course. On the other hand the curve A follows a S-shaped course

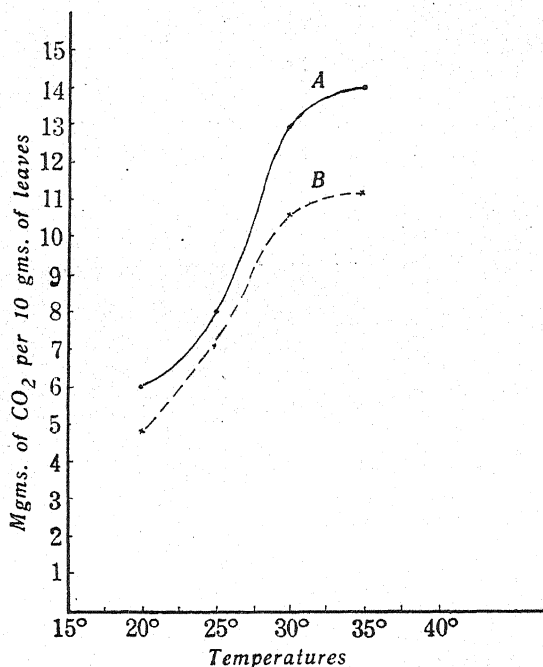


FIG. 7

indicating a slow rise between 20–25° C and 30–35° C ; between these two extremes the curve indicates a rapid acceleration.

#### THE $Q_{10}$ VALUES

The  $Q_{10}$  values for both actual assimilation and derived real assimilation are shown below :—

Temperature in °C	$Q_{10}$ for Actual Photo- synthesis	$Q_{10}$ for Derived real Photo- synthesis
20–30	2.2	2.16
25–35	1.53	1.75

My thanks are due to my old students Mr. N. L. Pal and Dr. U. N. Chatterji for their help in going through the manuscript.

#### SUMMARY

The photosynthetic rate in artificial light at different temperatures in leaves of *Eugenia jambolana* were estimated.

A new type of gas regulator has been devised which divides the gas equally while passing through two experimental chambers. The limit of accuracy reached is  $\pm 1$  c.c. in one hour.

It was shown in a previous paper that respiration rate in light increases; therefore it has been argued that in order to arrive at a truer index of photosynthesis the enhanced rate of respiration in light is to be taken into consideration. The derived values of photosynthesis thus obtained are shown at  $20^{\circ}$ ,  $25^{\circ}$ ,  $30^{\circ}$  and  $35^{\circ}$  C. The  $Q_{10}$  for derived real assimilation is highest between  $20^{\circ}$ – $30^{\circ}$  C being 2.16 while between  $25^{\circ}$ – $35^{\circ}$  it becomes lower, being only 1.75.

## STUDIES ON THE PHOTOCHEMICAL ACTION IN PLANTS

### III. The Influence of visible light on the rate of respiration of some coloured flowers

BY SHRI RANJAN

AND

BRIJ BEHARI LAL SAKSENA

Received for publication on August 30, 1940

#### INTRODUCTION

EXPERIMENTS on the effect of light on respiration have, in the majority of cases, been carried out with the non-green plants for the simple reason that in such cases the simultaneous effect of light on the photosynthetic process does not take place.

Day,<sup>1</sup> however, reported a 3 to 4% increase in the respiration of barley in diffuse day-light over the respiration rate in darkness, as measured by O<sub>2</sub> consumption and CO<sub>2</sub> production.

Ranjan<sup>3</sup> found an increase in respiration in the case of non-green leaves of Croton when exposed to artificial light.

Spoehr<sup>4</sup> explained the effect of illumination on respiration as due to air ionized by the sun's rays which gave a slightly greater rate of respiration than night air or deionized air.

Parija and Saran<sup>2</sup> working on the albino varieties of Aralia found that by exposing these plants, even to a short period of diffused light, the respiration rate after the exposure got augmented.

#### MATERIALS AND METHODS

The plant materials used in this work were the inflorescence of Bougainvillea (pink), Nerium flowers (yellow and deep pink), and Canna flowers (pale yellow).

In each case neither too young nor too old flowers were chosen. The green leaves from the smaller inflorescences of Bougainvillea were removed before experimenting.

Every experiment ran with a control and in every case care was taken that the control and the experimental sets were nearly of the same age and size.

As a source of light a 1,500 watt Osram bulb was used which was kept at a distance of 1 foot from the plant chamber.

The flowers were brought from the garden, washed with distilled water, divided into two sets, weighed and then each set kept in a respiratory chamber with some water.

The plant chambers were then put in a Hearson's incubator and the temperature regulated at 35°C.

For the determination of the rate of respiration, the method of estimating the amount of carbon dioxide given out by the flowers, in a current of air, was employed using Blackman's air commutator.

#### EXPERIMENTAL RESULTS

The first experiment of the series, on the Canna flowers, shows (Fig. 1) that the respiration rate of both the experimental and the control sets started nearly together. At the end of 18 hours light was given to the experimental set for a period of 8 hours. The respiration rate of the flowers during the period in light, shows a very slight increase. When the light as switched off, the respiration rate slightly dropped off.

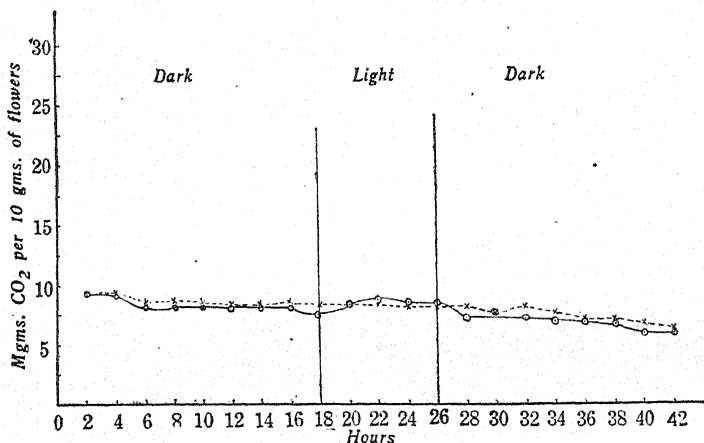


FIG. 1

Figs. 2, 3 and 4 give the respiration curves, of Nerium (yellow), Nerium (deep pink) and Bougainvillea (pink) respectively. In all

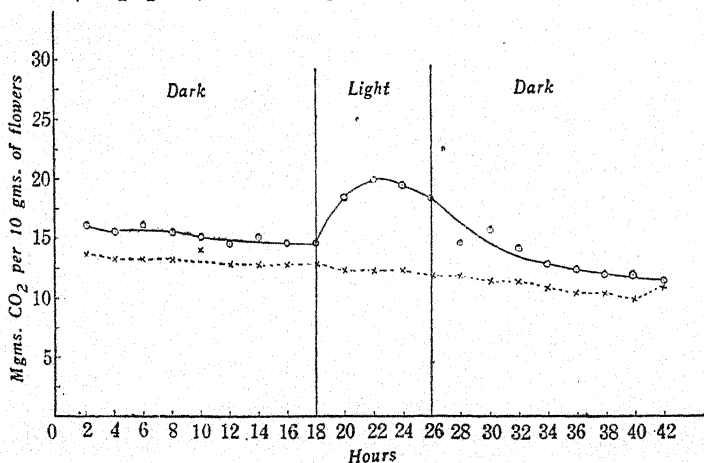


FIG. 2

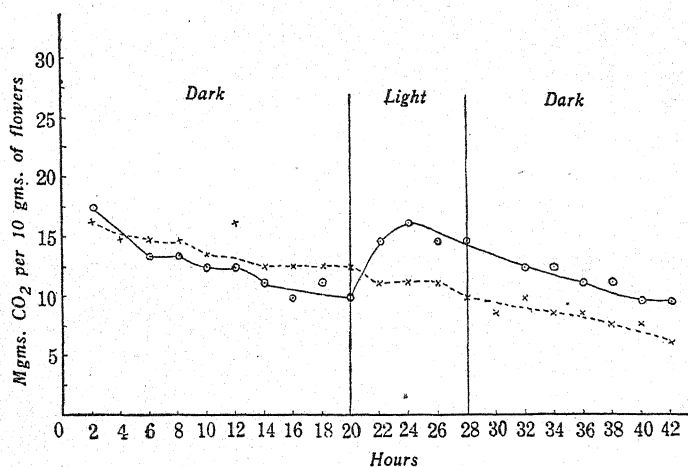


FIG. 3

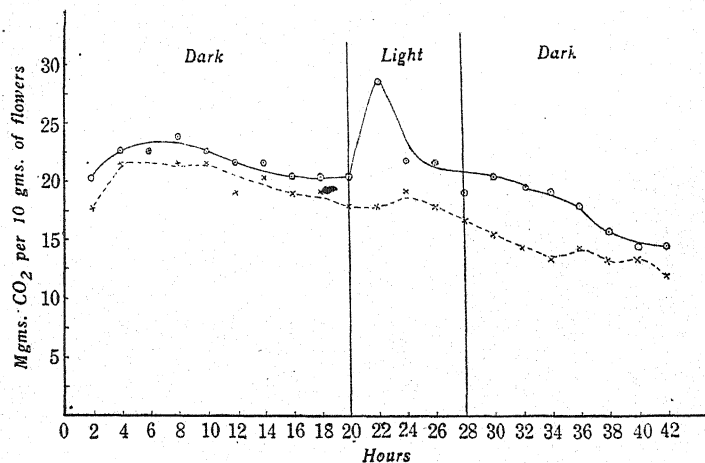


FIG. 4

these cases light was administered for eight hours, after a period of 18–20 hours in darkness.

Unlike the respiration rate of *Canna* flowers the respiration of *Nerium* and *Bougainvillea* showed a rapid and marked increase during the period the flowers were in light. When light was switched off, in all the cases, the respiration rate declined off.

#### THE CAROTINOID PIGMENTS AND ANTHOCYANINS OF THE FLOWERS

Only rough quantitative analyses of these pigments were undertaken with a view to find out whether there was any connection between these pigments and the light effect on respiration. In each case 5 gm. of flowers were taken which were ground to a

fine paste with a little acetone. 20 c.c. of 80% acetone was then added and the mixture transferred to a Buchner funnel. The filtrate was then transferred to a separating funnel and an equal volume of ethyl ether, and water was added to separate the fat soluble non-saponifiable carotinoid pigments and the water-soluble anthocyanins. Portions of each extract was then poured into test-tubes and the depth of colours compared. The results showed, that the *Canna* flowers contained the least quantity of both the carotinoid and anthocyanin pigments while the *Nerium* and *Bougainvillea* flowers contained relatively far more carotinoid and anthocyanin pigments.

#### DISCUSSION OF THE RESULT

*The floating respiration.*—According to the type of the fall, the floating respiration can be divided into two types viz., (1) The *Canna* type. Here the respiration rate starts low e.g., 10 mg. CO<sub>2</sub> per 20 gram. hours, and this low rate is maintained at a fairly constant level throughout the experiment. The colouration of the petals of these flowers show that the pigments are relatively less than in other type of flowers investigated. This coincides with the fact, that the respiration rate in light augments very feebly. It may, therefore, be safe to assume that where, normally, the floating respiration starts low and is a flat curve, the respiration in light will augment very feebly, if at all. The second type is the *Nerium* type. Here the floating respiration starts high and gradually comes down to a low flat level. The respiration of these plants, in light, shows a marked and rapid increase. Thus one may again assume that where the floating respiration normally shows a fall, the respiration in light will be markedly increased. The colouration of the flowers of the *Nerium* type shows deep pigmentation.

#### THE RESPIRATION IN LIGHT

That there is a clear co-relation between the depth of colouration of the flowers and the respiration rate, in light, is clearly shown by Figs. 1-4. In *Canna* where the colouration was slight, the respiration augmented very feebly. In the cases of *Nerium* and *Bougainvillea*, however, the increase is very marked and so also the depth of colouration of the flowers. The increase of the respiration in light over that in darkness shows that whereas in *Canna* it was 1.1 times only, in yellow *Nerium* it was 1.35, in pink *Nerium* 1.7 and in *Bougainvillea* 1.4 times. This clearly proves that light will increase the respiration rate only when suitable rays are absorbed by the respiring tissues of the plants.

#### THE RESPIRATION RATE IN DARK AFTER LIGHT

In the case of the *Canna* type the respiration rate in light which was only very slightly above the control set, falls at once to the level of the respiration in darkness. The *Nerium* type, however, shows a steady slow decline in the respiration rate in darkness after light. And it is only after 15 hours or thereabouts that it tends to

join the respiration rate of its control set. This forcibly points out that the after-effect of light does not damp out rapidly, but that it continues for several hours.

#### SUMMARY

The respiration rates, in light, of *Canna*, *Nerium* and *Bougainvillea* flowers have been studied.

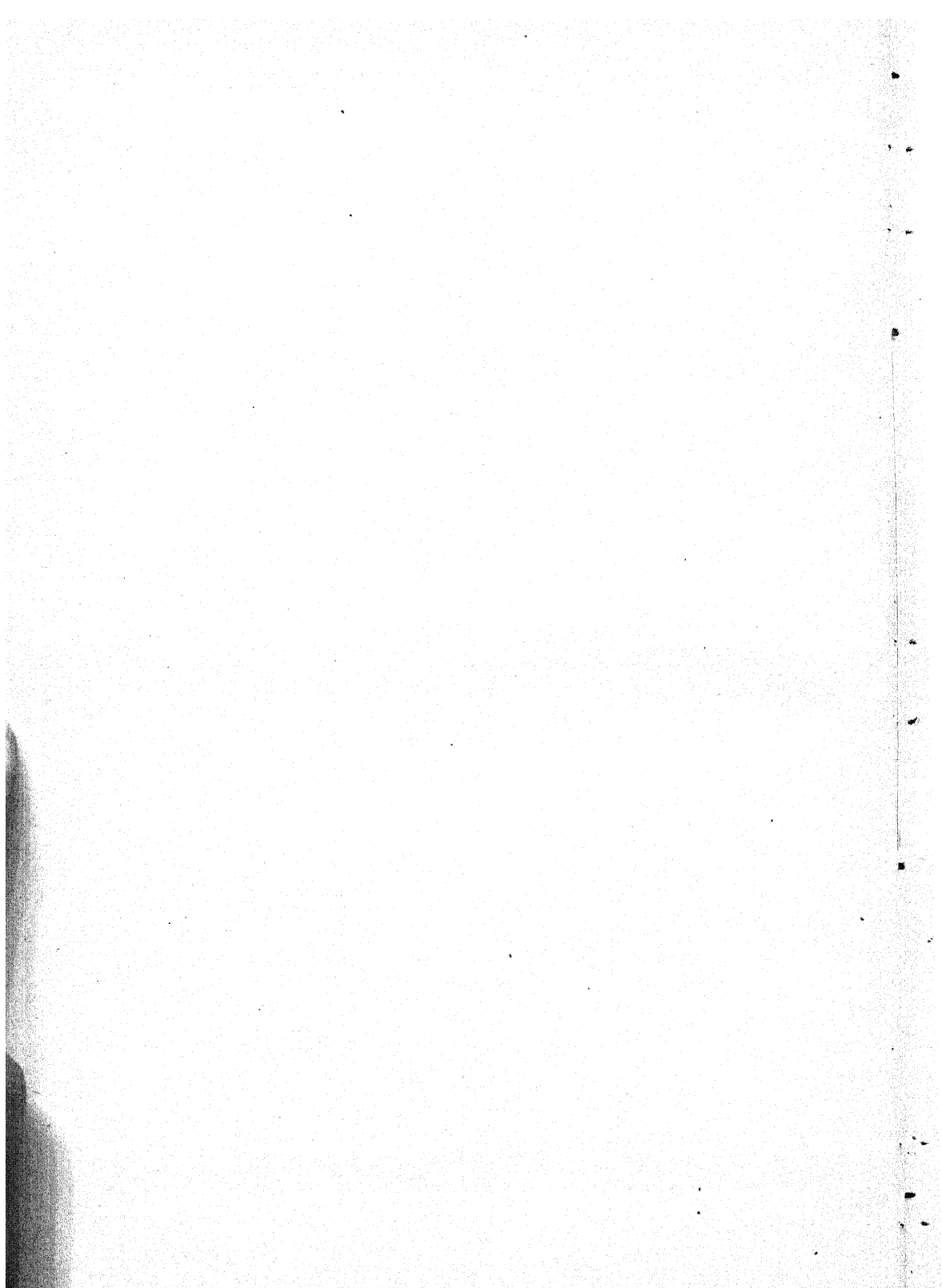
The respiration rate of *Canna* flowers showed only a very slight increase in light. On the other hand, the respiration rate of *Nerium* and *Bougainvillea* flowers markedly increased in light. This increase is correlated with the concentrations of the carotinoid and anthocyanin pigments.

It is suggested that these pigments by absorbing the necessary rays of the visible spectrum, cause an increase in the respiratory rate in light. It is further shown that the after-effect of light continues for several hours.

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## STUDIES ON THE PHOTOCHEMICAL ACTION IN PLANTS

### IV. The effect of violet and ultra-violet radiations on plant respiration

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Received for publication on August 30, 1940

#### INTRODUCTION

OF the relationship between respiration and ultra-violet radiations we have almost no knowledge. Masure<sup>5</sup> however, using mercury vapour lamp, with corning G. 586 A.W. screen, showed that the respiration is temporarily increased. Its effect disappears soon after it is discontinued. However, his experiments have been very few to warrant a generalization. Lindner<sup>3</sup> showed a remarkable acceleration of fermentation by the radiations from a mercury vapour lamp. He used a solution of 30 gm. of glucose in 300 c.c. of water which he inoculated with 5 gm. of pressed yeast. His results are shown in tabular form below.

TABLE I

Hrs. from start	CO <sub>2</sub> production in c.c.	
	Irradiated	Control
2	156	4
4	645	118
6	1,085	119

On the other hand there has been overwhelming evidence to show that ultra-violet radiations have a distinctly injurious effect upon the germination and early growth of seedlings. The same harmful effects have been proved on more mature plants by Arthur and Newell,<sup>1</sup> Popp and Brown<sup>6</sup> and others. Maquenne and Demoussy<sup>4</sup> have shown by plasmolytic tests that the epidermal cells of the leaf are killed by ultra-violet radiations, but that the palisade cells of the interior remain uninjured. Delf, Ritson and Westbrook<sup>2</sup> showed that the epidermal cells of the leaves of *Pelargonium* collapsed, which was followed by rolling and distortion of the

leaves after exposure to ultra-violet radiations for 2 minutes at a distance of 3 ft.

Thus to gain some more insight into the problem of the effects of these short-wave radiations on plant respiration the present work was undertaken.

#### METHODS AND PROCEDURE

For the determination of the rate of respiration the method of estimating the amount of  $\text{CO}_2$  given out by the leaves, in a current of air was employed. For this purpose the well-known air current commutator of Dr. F. F. Blackman was used.

The rectangular brass plant chamber containing excised leaves of *Eugenia jambolana* was kept in a Hearson's cool incubator and the temperature was normally maintained at  $25^\circ \text{C}$ .

*Light.*—As a source of light atmospheric type mercury vapour ultra-violet lamp was used.

*Sugar Estimation.*—Pavy's solution was employed for sugar estimation of the leaves.

#### EXPERIMENTAL RESULTS AND DISCUSSIONS

In the first two experiments light given to the plants was from an ultra-violet ray apparatus placed at a distance of about 1 ft. As light had to travel through two glass plates—one being the plant chamber and the other the window of the electric incubator—it is presumed that much of the short-wave rays was absorbed before reaching the plant surface. Thus the effect felt cannot be interpreted to be due to ultra-violet light, but should be looked upon as ordinary light with a greater proportion of shorter waves.

In Fig. 1 the result of exposing the leaves to such a light at a temperature of  $33^\circ \text{C}$  is given. The exposure was of a duration of

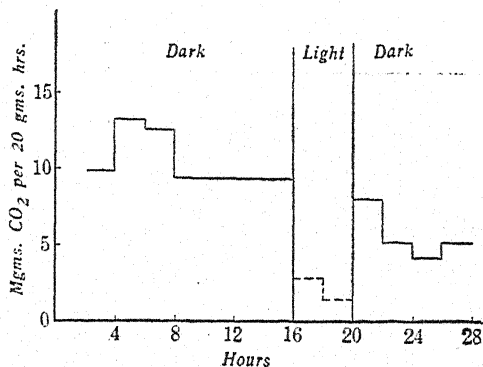


FIG. 1

4 hours after 16 hours of starvation in dark. The exposure causes the rate of the emission of  $\text{CO}_2$  to fall immediately to a very low level which again rises on switching off the light.

In the next experiment (Fig. 2) the same was repeated except that the leaves were kept at a temperature of 25°C instead of

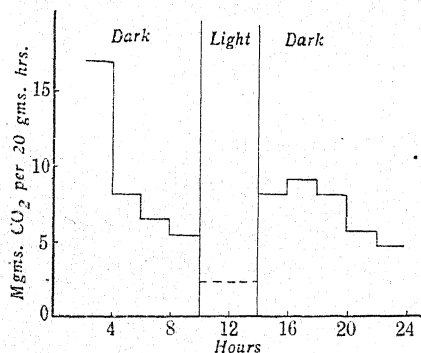


FIG. 2

at 33°C. In this experiment, as in the previous one, light causes the  $\text{CO}_2$  emission to fall to a very low level. However, unlike the first experiment, on switching off the light the respiration rate rapidly mounts up to reach a higher plane than that maintained before exposure.

The fall in the  $\text{CO}_2$  emission on exposure to light is due to the fact that part of the carbon dioxide given out in respiration was photosynthesised by light. The subsequent rise over the normal drift is due to the direct effect of light on respiration as has been previously shown by Ranjan.<sup>7,8</sup> The smaller effect, in the first experiment, strengthens the belief that at higher temperatures the direct effect of light is smaller than at about 25–27°C (see Ranjan<sup>8</sup>).

In the next two experiments the same source of light was used as in the previous cases but the light was made to pass through a jacket of methyl-violet solution (about 4" deep) of such a concentration that only violet rays could pass. Thus in fact the leaves were supplied with a monochromatic light of violet colour. In the first of these experiments (Fig. 3) light was given for a period of 6 hours.

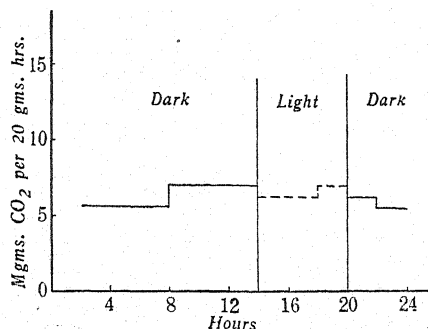


FIG. 3

The results do not show any marked variation. In the second experiment with violet rays (Fig. 4) light was given for short dura-

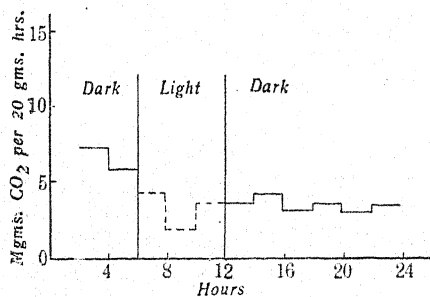


FIG. 4

tions of 5 minutes, every hour, though the respiration was estimated every two hours as before. The results are essentially similar to the one just described.

The main cause of the absence of any effect is the weak intensity of violet light falling on the leaves owing to the thick layer of the solution. Thus the effect of violet light, if any, cannot be gauged from these experiments and more investigations are needed with stronger light before any definite conclusions can be reached.

In the next three experiments, however, there was a change in the treatment and the leaves were taken out of the plant chamber and exposed to direct light from the mercury vapour lamp from a distance of 3 ft. so that there could be full play of the shorter waves on the leaves.

In the first experiment of this series (Fig. 5) after noting the respiration for 4 hours, light was given for 8 minutes and then again

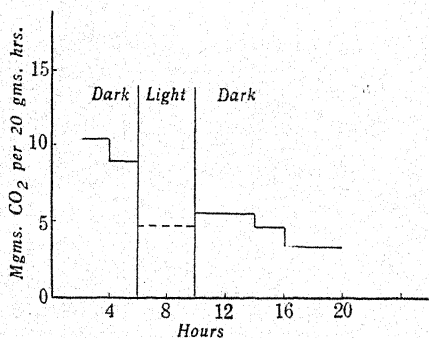


FIG. 5

after 2 hours for 10 minutes. It would be seen that from a rate of 9.0 mgm. CO<sub>2</sub> per 20 gm. hours, the respiration falls to a low value of 4.9 mgm. just after each exposure to light. However in continued

darkness the respiration improves to reach 5.6 mgm. After reaching this value the respiration rate again falls off.

It is necessary at this place to note that in this experiment when the leaves were exposed directly to ultra-violet light, they, just after exposure, developed a faint odour. At first the smell was mistaken to be due to ozone, produced in the air by ultra-violet light but careful examination revealed that though it somewhat resembled the smell of ozone yet it was of a different character.

This experiment thus shows the adverse effect of ultra-violet rays on plant respiration. In these experiments the period of exposures to light was too small to have any photo-synthetic effect.

In the next two experiments, a greater number of leaves were taken and hourly respiration was estimated instead of two-hourly ones as in the previous experiments.

Light which was given to the plants in the 5th and 7th hours (Fig. 6) was of very short durations (3 minutes at a distance of 3 ft.).

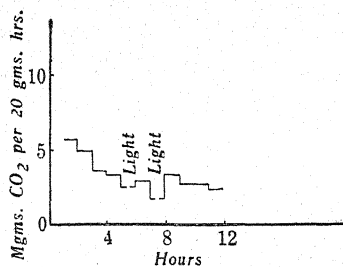


FIG. 6

The results however, definitely established the harmful effect on respiration. Just after the first exposure the respiration falls from 3.1 to 2.3 mgm. CO<sub>2</sub> and during the next period rises to 2.9 mgm. when a second exposure is made. This second exposure has a greater effect and the rate falls to only 1.6 mgm. which again rises in later periods and reaches the original level.

In the last experiment in order to reduce the dose of ultra-violet light the source of light was kept at a distance of about 6 ft. from the plants. The result (Fig. 7) shows that even minute doses

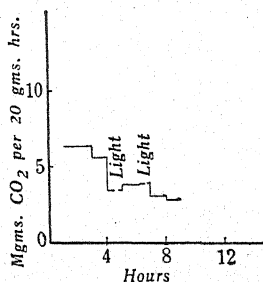


FIG. 7

have the same inhibitory effect on the respiration rate though it is smaller. As a result of the exposure of 3 minutes the respiration falls from 5.6 mgm. to 3.5 and this again rises to 3.9 which, however, keeps constant even on second exposure. The rate, however, subsequently falls.

Finally, the percentage of the reducing sugars of the exposed and unexposed leaves were undertaken. The results are given below.

TABLE II

Treatment	Sugar content	
	Experimental %	Control %
15 Minutes exposure to ultra-violet light at a distance of 1 ft. .. ..	0.623	0.619
Two exposures of 3 minutes each at an interval of 2 hours at a distance of 6 ft. ..	0.41	0.41

It is evident from the above table that exposures, to either strong or weak concentrations of the ultra-violet rays, have no effect upon the reducing sugars of the leaves.

## SUMMARY

(1) The effect of light from an atmospheric mercury vapour lamp on plant respiration was studied. It was found that owing to a great reduction in ultra-violet part of radiation, due to glass surfaces through which the light passed, and owing to the presence of other rays in the light the effect was more or less similar to the effect of ordinary light on respiration.

(2) When exposed to rays passing through methyl-violet solution, so as to give monochromatic violet light, the respiration rate does not show any appreciable change.

(3) On exposure (8 minutes and 10 minutes at an interval of 2 hours) to direct light from this apparatus at a distance of 3 ft. containing a much greater proportion of ultra-violet light the respiration shows a fall which slightly rises on switching off the light. Shorter exposures also (3 minutes from a distance of 6 ft. at an interval of 1 hour) clearly proves the inhibitory effect of ultra-violet radiation on respiration.

(4) Sugar estimations show that there is no change in the reducing sugars after exposures to either strong or weak ultra-violet light.

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ON THE MORPHOLOGY AND CYTOLOGY OF  
*EUDORINA INDICA* IYENGAR\*

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(Communicated by M. O. P. Iyengar)

Received for publication on June 10, 1940

*Eudorina indica* Iyengar was first collected by Dr. M. A. Sampathkumaran of Bangalore in a rain-water pool at Talguppa in Mysore Province and later on by Prof. M. O. P. Iyengar in a similar situation at Madras. Since then, there has been no other record of this alga anywhere. During the South-West monsoon of 1937, this alga came up in large numbers along with other algae, especially members of the Volvocaceae, in a rain-water pool at Madras, forming a sort of water-bloom. A good account of the alga has been given by Iyengar (1933, pp. 339-43), but his description was based on material preserved in formalin. The occurrence in plenty of the living material of the alga was taken advantage of to make a detailed study of its life-history and cytology at the kind suggestion of Prof. Iyengar.

## MATERIAL AND METHODS

The life-history of the alga was followed in the field as far as possible and also in cultures kept in the filtered pool water in glass vessels in the laboratory. Mainx's Volvox solution (Gross, 1931, p. 210) and Uspenski and Uspenskaja's solution (Uspenski and Uspenskaja, 1925, p. 307) were employed for the cultures.

For cytological studies the living material both from the field and from the laboratory cultures was fixed when the colonies were showing signs of division. The material was fixed at regular intervals of half an hour each both during the night and the day time. Division took place mostly during nights between 9 P.M. and 2 A.M. and abundant division figures were obtained in material fixed between 10 and 11 P.M. The following fixing fluids were used—Flemming's weak, Flemming's strong, Flemming's strong diluted with an equal amount of distilled water, Schaudinn's sublimate acetic-alcohol (5% acetic), Nawaschin's fluid, Bouin's fluid and Allen's modification of Bouin's fluid (PFA<sub>3</sub>). Of these the best results were obtained in material fixed in Flemming's mixtures and in Schaudinn's fluid.

The material fixed in Flemming's fluids was washed thoroughly in water and run up to 70% alcohol. The material fixed in

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This paper formed part of a thesis submitted for the Degree of Master of Science of the University of Madras.

Schaudinn's fluid was washed in several changes of 50% alcohol and was freed from traces of mercuric chloride by treatment with Lugol's iodine solution. Smear preparations were made from the material in 70% alcohol according to protozoological methods (McClung, 1937, pp. 530-31). A drop of the alcohol containing the material is pipetted over an albumen-smeared slide and spread on it with the aid of a cover glass. When the alcohol has very nearly evaporated, the slide is immersed in a jar of 80% alcohol. Then the slides are brought down the alcohol grades to water before they are stained. Smear preparations were also made directly from the living material. A drop of the water containing the living material is placed on an albumen-smeared slide and then fumed over osmic vapour for half to one minute. The slide is allowed to dry up completely. It is washed in water and bleached in dilute hydrogen peroxide to remove the blackening effects of osmic vapours before it is stained. Smear preparations were also made in the following manner. The smeared living material is fixed by pouring over them killing fluids by means of a pipette. After drying, these slides are treated as above. All these three methods of smear preparations proved good. Microtome sections also were made from material imbedded in paraffin. The changes through the alcohol and the xylol grades were made by decantation, since centrifuging tended to break up the colonies. The sections of material fixed in Flemming's fluids were bleached either in dilute hydrogen peroxide or a solution of chlorine in 30% alcohol before staining.

A number of stains was used besides Heidenhain's iron-alum hæmatoxylin, such as, safranin and light green, Biondi's triple stain and Mayer's hæmalum. The hæmatoxylin stain proved to be the most satisfactory, though fairly good results were also obtained from material stained in safranin and light green and also in Mayer's hæmalum. The nuclei are generally rather difficult to stain, but a treatment for 6-12 hours in 1% chromic acid solution followed by a thorough washing in running water for about half to one hour before mordanting in iron-alum proved very effective in making the nucleus take the stain. The slides were left in 4% iron-alum for two hours and then stained in  $\frac{1}{4}$ % hæmatoxylin for 2-4 hours. Destaining was carried out in a saturated aqueous solution of picric acid. After washing in running water, the slides were treated for 1-2 hours with 1% aqueous solution of sodium acetate to remove any traces of acid that may still remain and then thoroughly washed in running water again. After dehydration, the slides were cleared in clove oil and xylol and mounted in neutral canada balsam.

Inversion stages of daughter colonies were observed from living material in hang-drop cultures. For making drawings or photographs of dividing colonies and of inversion stages, the living material had to be kept more or less stationary for some time. For this purpose, the colonies were mounted under a cover glass. In order to prevent the cover glass from pressing and injuring the colonies too much, pieces of broken cover glass were introduced

between the cover glass and the slide. This enabled one to follow all the stages with ease.

#### DESCRIPTION OF THE COLONY

The colonies of *Eudorina indica* are ellipsoidal to sub-globose and often egg-shaped. Many colonies showed a broad anterior end and a narrow posterior portion. In these latter colonies the small front cells were situated slightly apart, while the large cells of the narrower posterior region were crowded together (Text-fig. 2).

The normal colony of *Eudorina indica* contains 64 cells arranged in seven definite tiers. The first and the seventh tiers have four cells each, while the second has eight cells and the remaining tiers (third to sixth) have twelve cells each (Text-fig. 1). The cells of the first two tiers are much smaller than those of the remaining tiers, but the cells of the second tier are larger than those of the first. In the remaining tiers a gradual increase in size of the cells is seen from the anterior to the posterior end. The cells of the seventh tier, however, are generally slightly smaller than those of the sixth. The following table gives the average measurements of the cells of the different tiers:—

I tier	..	..	10.1-12.3 $\mu$
II tier	..	..	14.3-16.3 $\mu$
III, IV & V tiers	..	..	16.2-24.5 $\mu$
VI tier	..	..	22.4-25.0 $\mu$
VII tier	..	..	20.4-22.4 $\mu$

Mixed with the 64-celled normal colonies were often found 32- and 16-celled colonies of the alga. The 32-celled colonies resemble those of *Eudorina illinoisensis* (Kofoid) Pascher but in *E. indica* the cells of the first two tiers are smaller than those of the rest, whereas in *E. illinoisensis* the cells of only the first tier are smaller (Text-fig. 3).

The description given above agrees very well with that given by Iyengar (1933) except for some slight differences in the dimensions of the colony and the cells. The slightly smaller dimensions given by Iyengar are evidently due to his material having been preserved in formalin for some time, while the dimensions given in the present paper were all taken from living colonies.

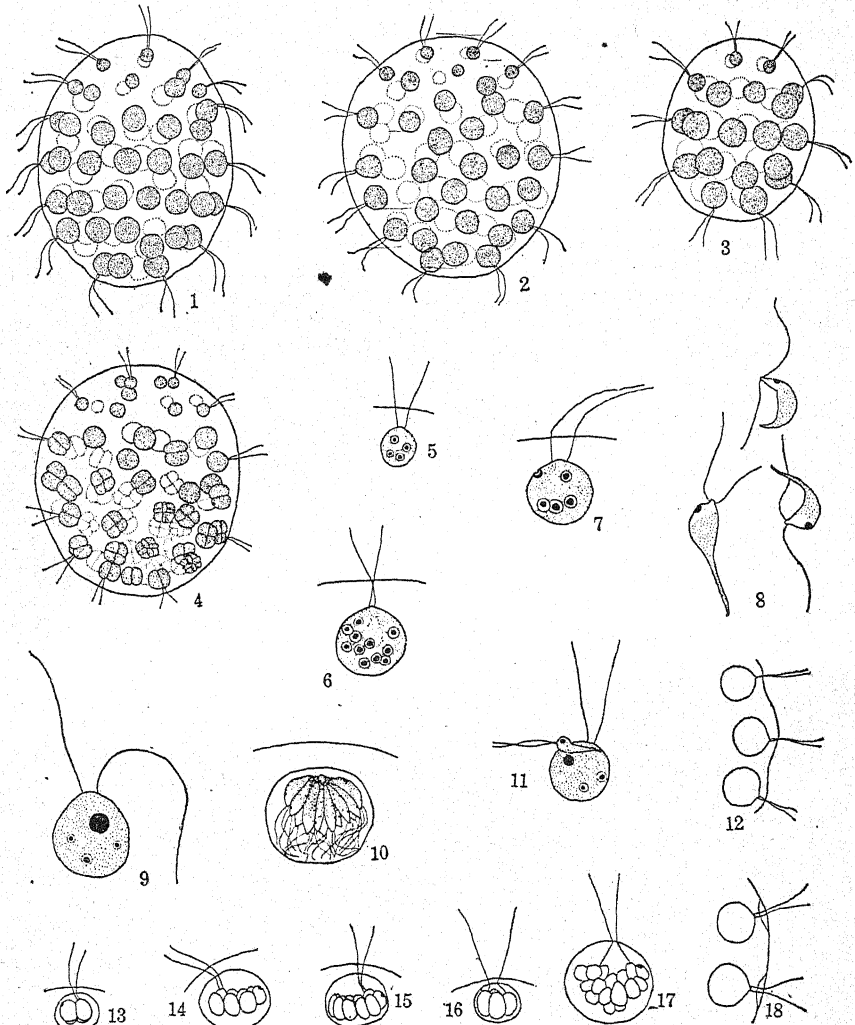
The colony exhibits a marked polarity and moves forward rotating on its longitudinal axis, one end of the colony, the anterior end, being always directed forwards during the movement. The rotation is both clockwise and anti-clockwise in the same individual. It moves rotating in one direction for sometime and then pauses for a while, and then proceeds forward again rotating in the opposite direction. But the change in rotation may take place without any pausing also. Many other observers have noted this rotatory forward motion of the colony in *Eudorina*. Carter (1858, p. 238), in the living specimens of *E. elegans* collected at Bombay, observed rotation in both directions. Kofoid (1898, pp. 282-83) made the same observation in *E. illinoisensis*. Grove (1915) found colonies

of the same species rotating clockwise unless their movement was obstructed in some way, when they receded rotating in the opposite direction. Iyengar (1933, p. 330) found that the colonies of *E. elegans* rotated mainly in a clockwise direction, but quite as often rotated in the opposite direction. He found, however, a few stray colonies moving forward without any rotation whatever. Such a movement was not observed in this alga in any of the colonies, though numerous living specimens were examined.

The colonies are definitely phototactic. The living material kept in a petri dish or a watch glass, soon crowd towards the illuminated side of the glass vessel imparting a bright green colour to that side. When this side is viewed under the low power of a microscope, it is seen that the anterior ends of all the colonies are directed towards the source of light. Two petri dishes containing active, healthy material were placed on either side of an electric light in a dark room. The material was kept for a time in the dark before the light was switched on. At first the organisms were evenly distributed in the water giving it a uniform light-green colour. But after a time all the colonies in each vessel were found to have moved towards the side nearest the light making that edge of the petri dish intensely green. Now the single light between the two petri dishes was removed and two such similar lights were placed outside the pair of petri dishes at an equal distance from the dishes. After a little time the colonies in each of the dishes were found to have moved to the newly lighted side of the dishes. When exposed for a long time to bright light, the colonies seem to lose their phototactic sensitivity and to show "fatigue". They all go down to the bottom of the dishes and show no definite reaction to light. At night, the majority of the colonies generally settled down at the bottom of the dish, only those that were dividing being seen as a floating scum at the surface.

*The Cell.*—The smaller cells of the front two tiers are slightly ovate in shape while the cells of the posterior ones are more or less globose, though often somewhat flattened at the anterior end. The protoplast when highly magnified appears to be slightly beaked in front.

Each colony is surrounded by two mucilaginous coats, the inner one being denser and firm, the outer coat is less dense and diffuse. This outer mucilaginous envelope is generally not well seen in material preserved for some time in formalin as it appears to become dissolved in the preserving fluid. The wall of each individual cell has two layers, the outer one being thick and gelatinous, while the inner one is firm and thin and closely invests the protoplast. When the colonies are treated with dilute aqueous methylene blue, hexagonal reticulations are seen round the cells. These reticulations represent the outer edge of the thick gelatinous layer of the cell-wall. Kofoid (1898) and Conrad (1913) observed these hexagonal reticulations. Grove (1915) remarks that he could not succeed in demonstrating any hexagonal reticulations round the



Text-Figs. 1-18. *Eudorina indica* Iyengar. Fig. 1. A normal colony ( $\times 215$ ). Fig. 2. A colony with obovate shape ( $\times 215$ ). Fig. 3. A 32-celled colony ( $\times 215$ ). Fig. 4. A colony showing the posterior cells dividing ( $\times 215$ ). Fig. 5. An anterior cell with four pyrenoids ( $\times 676$ ). Fig. 6. A posterior cell with eleven pyrenoids ( $\times 676$ ). Fig. 7. A cell from the fourth tier showing eyespot ( $\times 676$ ). Fig. 8. Spermatozooids ( $\times 880$ ). Fig. 9. Egg-cell with two cilia ( $\times 880$ ). Fig. 10. A sperm bundle ( $\times 676$ ). Fig. 11. A spermatozoid attached to the egg ( $\times 465$ ). Fig. 12. Portion of a colony viewed from the side showing gelatinous wall bulging opposite each cell ( $\times 324$ ). Figs. 13-15. Plaquea with the two cilia of the mother colony still attached to one of the cells. The eyespot of the mother-cell is seen in another cell ( $\times 324$ ). Figs. 16-17. Two stages of the plaquea in *E. elegans*. The two cilia of the mother-cell are attached on two different cells of the plaquea (from drawings by Iyengar). Fig. 16 ( $\times 220$ ). Fig. 17 ( $\times 324$ ). Fig. 18. Portion of colony viewed from the top showing depression of the gelatinous envelope opposite each cell. Note the separate insertion of the cilia ( $\times 324$ ).

cells, until he adopted the expedient of pressing upon the cover glass, so as to expel the cells. This reticulate appearance is considered by him as an artefact produced by the mutual pressure of the gelatinous capsules surrounding each cell at a certain stage. Iyengar (1933, pp. 331 and 336) found these reticulations very distinctly both in *E. elegans* and in *E. illinoisensis*. In this connection, he states that "the outermost edge of the gelatinous layer takes up the stain quicker than the rest of the wall, and hence the more or less polygonal contour stands out rather prominently for some time. Later on, when the stain becomes more uniformly distributed, the reticulation is not so distinct. The reticulation is thus really due to mutual pressure of the gelatinous walls of the cells upon one another as Grove suggests, but it is not clear why he regards it as an artefact".

The firm thin inner membrane of the cell next to the protoplast consists of cellulose. This layer turns blue when treated with iodine and sulphuric acid. When the colonies are treated with ruthenium red, the general gelatinous envelope of the whole colony and also the outer gelatinous layer of the individual cells takes up a rose colour indicating the hemicellulose nature of the mucilage. But since both pectic and non-pectic hemicelluloses become stained with ruthenium red, the following test (Rawlins, 1933, p. 42) was made to distinguish the pectic from the non-pectic portions. The material was kept in 4% carbonate free sodium hydroxide solution for various periods, some for 3 hours, others for 4 hours and some others again for 24 hours. Afterwards it was washed free from the alkali and acidified with dilute acetic acid and stained with ruthenium red (1 : 1000). In material kept in caustic soda for a long time, say for 24 hours, all the non-pectic hemicellulose portions would be completely dissolved, only the pectic portions being left behind. But if the material is treated for only a short time in caustic soda, say for 3-4 hours only, a certain quantity of non-pectic hemicellulose matter would still be present along with pectic material. In the present alga, in the material treated for 24 hours, only the general mucilaginous envelope of the colony is left behind as a thin layer, the rest of the mucilaginous portions being dissolved out by the treatment. But in the material treated for only 3-4 hours the general envelope of the colony was not reduced to such a thin layer but was slightly broader, and a small quantity of mucilage was still left inside the envelope. This shows that the general mucilaginous envelope of the colony consists of two portions, a thin outermost pectic layer and a broader inner non-pectic portion. As regards the thick mucilaginous layer round each individual cell, this gets dissolved out by the treatment with caustic soda for 24 hours; but when treated for only 3 or 4 hours a small portion of this layer is still present. This shows that the gelatinous layer of the individual cells are non-pectic in nature.

*Cilia*.—Each cell has two cilia which are twice the length of the cell or slightly longer. They are situated at the apex of the cell slightly separated from each other (Text-fig. 18). The line



connecting the two cilia of a cell is more or less at right angles to the plane of the longer axis of the colony. When the colony is lying on its sides the two cilia overlap and are therefore not seen separately (Text-fig. 12). On the other hand, when the colony is viewed from its anterior or posterior end, the two cilia are seen standing apart in almost all the cells of the colony (Text-fig. 18). Conrad (1913) described the two cilia of *E. elegans* as coming out of a single tube-like aperture (Pascher, 1927, Fig. 395). Akehurst (1934) found the two cilia coming out of two separate funnel-like tubes. In the present alga the two cilia emerge through two separate tubular passages in the general mucilaginous envelope.

In some colonies a careful examination showed that the mucilage is slightly depressed just in front of each cell at the point of exit of the cilia (Text-fig. 18). Hartmann (1921, Tab. I, Figs. 3 and 4) and Iyengar (1933, p. 331, Text-fig. 2 D) found the same to be the case in *E. elegans*. But Iyengar found that in some other colonies the outer mucilage was not depressed in front of the cells, but, on the other hand, was bulged out in front of each cell. Such a condition was found in a few colonies of the present alga also. In these colonies the gelatinous matrix is slightly bulged outside each cell, so that the boundary of the mucilage appears to follow the wavy contour of the cell (Text-fig. 12).

*Eye-spot*.—Each cell has an eye-spot which is concavo-convex in shape when looked at from the side, the convex side facing the inner portion of the colony and the concave side facing the outer (Text-fig. 7). As in the other members of the colonial Volvocales, the anterior cells have the largest eye-spots, the size of the eye-spot decreasing gradually in the posterior cells. The following are the dimensions of the eye-spot in the cells of the different tiers of a single full grown colony:—I tier  $3\mu$ ; II tier  $2.5\mu$ ; III tier  $2\mu$ ; IV tier  $1.5-1.8\mu$ ; V, VI and VII tiers about  $1.4\mu$ .

*Contractile vacuoles*.—Two contractile vacuoles are seen in the anterior region of each cell at the base of the cilia. They are situated in a plane at right angles to the plane in which the two cilia are situated, so that when viewed in position in which both the cilia are seen separately, only one vacuole is seen, and *vice versa*.

*Chloroplast*.—The chloroplast is cup-shaped with its concavity turned towards the anterior side. It almost completely fills the cell and the opening of the cup is very narrow.

*Pyrenoids*.—A number of pyrenoids is seen in the chloroplast, the number often reaching as many as 12 or 13. The pyrenoids are smaller in number in the cells of the anterior portion of the colony (Text-fig. 5) and increase in number in the cells situated more posteriorly (Text-fig. 6). The following gives an idea of the number of pyrenoids in the cells of the different tiers:—

I and II tiers	..	..	2-4 pyrenoids
III tier	..	..	5-6 "
IV to VII tiers	..	..	8-13 "



The different pyrenoids of the same cell vary in size, the largest often measuring  $4.2\ \mu$ .

In the living material the nucleus is not seen because of the intensely green and thick chloroplast which surrounds it. But in material properly fixed and stained each cell shows a single nucleus situated in the endoplasm enclosed by the hollow of the cup-shaped chloroplast. The nucleus is approximately  $4-5\ \mu$  in diameter and is spherical, but in some cases it appears slightly elongated. It contains a large deeply staining nucleolus. The rest of the nuclear space ("the outer nucleus") is clear, the chromatin network taking only a faint stain. At first much difficulty was experienced in making the nucleus take the stain in fixed material. According to Belar (1926) the resting nucleus in a number of Protophyta are characterised by a masking of the chromatin. Thus the chromatin is not distinguishable from the caryolymph even after staining, and the outer nucleus appears clear and homogeneous. But this difficulty in staining the outer nucleus was overcome, as mentioned earlier in the paper (see p. 114) by a pretreatment of the fixed material with 1% chromic acid solution in water, before staining.

*Neuro-motor apparatus.*—Two fine rhizoplasts are observed in stained material, passing from the nuclear membrane to the two basal bodies at the place of the emergence of the cilia (Text-fig. 29). Two rhizoplasts were observed in *E. elegans* by Hartmann (1921), in *E. illinoisensis* by Hovasse (1937) and in *Volvox* by Zimmermann (1921, p. 262).

#### ASEXUAL REPRODUCTION

Dividing colonies were not found in the material either in the field or in the laboratory at the beginning of the observations. Mature colonies were collected and kept under observation for several days both during nights and during the day time without any division being seen in any of them. Only occasionally was division seen in a stray colony or two. After remaining in a vegetative condition for nearly a month, the colonies began to show signs of division. After that, dividing colonies were seen very commonly in the water and almost all the mature colonies divided to form daughter colonies. The division generally started at about 9 P.M. and was very active throughout the night. But dividing colonies were observed during day time also in the field as well as in the cultures kept in the laboratory. Usually reproduction sets in only when the colonies have attained their maximum size and are intensely green. The cells then have many pyrenoids. In mature colonies division generally starts at the posterior end of the colony and proceeds in the anterior direction (Text-fig. 4; Pl. II, Fig. 3). The cells situated at the posterior end generally complete the daughter colony formation earlier than the ones more anteriorly situated. All the cells form daughter colonies excepting the front twelve cells which remain undivided. Iyengar (1933, p. 341-43) found only one dividing colony in his formalin material. In this single colony he found that the front twelve cells were not dividing

while the remaining cells were undergoing division. He states "unfortunately little is known about its reproduction, but the one case observed indicates that the front twelve cells do not divide, while most of the others do so. It is possible that in this alga the front twelve cells are truly somatic". My observations made on hundreds of dividing colonies of *E. indica* fully confirm Iyengar's suggestion that the front cells are somatic.

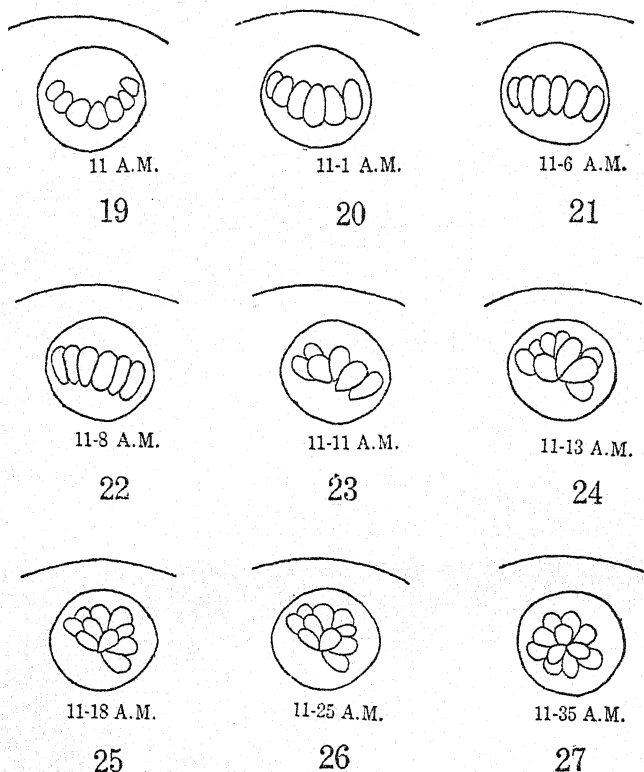
In the cell about to divide the protoplast contracts and draws away somewhat from the wall at the sides leaving a small space between the wall and the protoplast. The thin inner layer of the wall of the mother-cell becomes somewhat gelatinised, but continues to be intact and forms a vesicle round the developing daughter colony. It becomes gradually large enough for the completed daughter colony to rotate inside it.

The first division of the cell is longitudinal, resulting in two cells. The second division is also longitudinal but at right angles to the plane of the first division. The third division results in an 8-celled plate with the central four cells arranged in the cruciate manner characteristic of all colonial Volvocales. Each cell of this 8-celled plate divides into two and a 16-celled plate is formed. During this division the four central cells divide into four inner and four outer cells. Each of the four corner cells divides in a plane which is parallel with the division plane of one of the cross cells but at right angles to the division plane of the other cross cell adjoining it (Text-fig. 40; Pl. III, Fig. 17). Each of the cells of the 16-celled plakea then divides and the whole forms a curved plate. Goroschankin (1875, p. 29) states that in *E. elegans* the 32-celled stage does not arise by the division of all the sixteen cells of the previous stage. He says that the central four cells of the cross remain without division and represent the future anterior cells, while the corner cells each forms three cells by division and the other cells each form two cells and thus a 32-celled stage is obtained. In the smear preparations of the present alga a young developing daughter colony shows clearly sixteen metaphase plates (Text-fig. 41; Pl. III, Fig. 18) showing that all the cells of the 16-celled plakea divide to form the 32-celled stage. During the next division the 64-celled stage is reached.

#### INVERSION

Even at a very early stage, (*i.e.*, after the 16-celled stage), the until now flat plakea begins to get slightly curved and saucer-shaped. This curving continues further resulting in a hollow bowl-shaped plakea with the concavity directed towards the exterior of the colony (Text-fig. 19). This bowl-shaped plakea is still situated inside the vesicle of the mother-cell. The hemispherical plakea now begins to curve in the opposite direction and undergo a process of inversion. In this inversion process, the curved hemispherical bowl begins to gradually curve backwards and finally becomes quite flat. And, since the process of the curving backwards continues, the flat plakea becomes curved in the opposite direction

with the result that its convex side becomes finally directed towards the exterior of the mother colony and its concave side towards the interior of the colony. The curving does not stop now, but continues still further till the plakea becomes a completely closed structure (Text-fig. 27). During the closure of the open end of the



Text-Figs. 19-27. *Eudorina indica* Iyengar. Stages in the inversion of the daughter colony ( $\times 622$ ).

colony, the cells appear to become rapidly clustered together in a somewhat irregular manner without leaving much space in the centre. It is only sometime after this that the cells become more regular and arranged into a more or less spherical colony.

Inversion brings about certain changes in the orientation of the cells in the plakea. In the bowl-shaped daughter colony about to invert, the cells are so oriented that the hyaline anterior ends of the cells where the nuclei are situated are directed towards the concave side of the plakea and the posterior ends with the green chloroplast are directed towards the exterior of the bowl. As a result of the inversion the hyaline anterior ends of the cells of the plakea become directed to the exterior of the bowl and the posterior

ends of the cells with the chloroplast directed towards its interior. This is very clearly seen in microtome sections of inverting daughter colonies, where the nuclei and pyrenoids are well stained. In the bowl-shaped plakea before inversion the nuclei are seen at the ends of the cells pointing towards the inside of the bowl, while the pyrenoids are at the ends directed towards the exterior. After inversion the nuclei are seen at the ends of the cells directed towards the exterior of the bowl, while the pyrenoids are seen at the ends directed towards the interior of the bowl.

In the case of *Volvox* the number of cells is great and the young daughter colony about to invert assumes the form of a hollow sphere, with a narrow, small opening or phialopore (Pascher, 1927, p. 45, Fig. 421; Pocock, 1933, p. 37, Figs. 2*k* & *l*). The whole colony has to turn inside out through this very narrow phialopore and the process of inversion is therefore much complicated. In the case of *Eudorina* the broad opening of the hemispherical cup-shaped daughter colony about to invert represents the phialopore and since this is very broad, the process of inversion is very simple. But the process of inversion and the changes brought about by it are quite similar to those of *Volvox*, the difference being only one of degree and not of kind.

The process of inversion was first recorded in *Volvox* by Powers in 1908, but the complete process was first observed in living material by Kuschakewitsch in 1923 and later on by Zimmermann (1925) and Pocock (1933). Hartmann (1924, p. 378) was the first to describe the process of inversion in *Eudorina*. Merton in 1908 noticed a change in the polarity of the cells during the formation of young colonies in *Pleodorina illinoisensis*. But he did not recognise the fact that this change in polarity of the cells was due to the inversion of the daughter colony. He merely states that shortly after the sphere has closed there takes place in every cell a change of elements, and that, after this change, the hyaline pole is not towards the hollow of the daughter colony but towards the outer side (Merton, 1908, Taf. XXVII, Fig. 5). In his earlier paper, Hartmann (1921, pp. 232-33) describes the change in the polarity of the cells of the daughter colony before the cilia are formed, but is not sure whether the changes are brought about by the cell as a whole or by parts of it. In his second paper on *E. elegans*, he (Hartmann, 1924) recognised from a study of the living material that the change in the polarity of the cell of the colony is due to inversion. He gives a good account of this process in the paper. The author found that in *E. indica* also inversion takes place during daughter colony formation. His observations of this process in *E. indica* agree very closely with those of Hartmann made on *E. elegans*.

#### TIME OF INVERSION

Inverting daughter colonies were observed during nights as well as during day time. The whole process of inversion takes only a very short time and is completed within 30-40 minutes. The details as observed in the case of one single inverting daughter

colony is given below. The time taken by the plakea of this inverting colony for changing from its bowl-shaped condition (Text-fig. 19) to a flat condition (Text-fig. 21) is six minutes (11 A.M.-11-6 A.M.), and for the flattened plakea to become inverted backward (Text-fig. 24) is seven minutes (11-6 A.M.-11-13 A.M.). And another 22 minutes (11-13 A.M.-11-35 A.M.) was taken for the plakea to close up into a young colony (Text-fig. 27). So altogether the time taken for the whole process was 35 minutes. Hartmann (1924, p. 378) found that, in the case of *E. elegans*, it took a colony 1-1½ hours to complete this process. But he used material from agar cultures, whereas the observations recorded in the present paper were made from material collected fresh from the field. The inversion can easily be missed due to the shortness of the time taken by the process.

#### CILIA AND EYE-SPOT

During daughter colony formation the two cilia of the mother-cell of the colony remain attached to one of the peripheral cells of the developing plakea. The two cilia can be traced inwards from the surface of the mother-wall to one of the cells of the plakea. Before the mother-cell begins to divide, the protoplast first contracts somewhat away from the wall at the sides. When the protoplast divides the innermost wall layer as already pointed out, (p. 121), also expands a little through gelatinisation, and the space between it and the dividing protoplast increases. With further division, the space between the old mother-wall and the developing plakea becomes still greater. But the connection between the two cilia of the original mother-cell and the dividing protoplast is maintained throughout the development of the plakea. The two cilia of the original mother-cell can be seen attached to one of the cells of the plakea (Text-figs. 13-15). The cilia portion in the empty space between the old mother-wall and the plakea is evidently formed by the dividing protoplast as it contracts away from the wall. Merton has figured this in the case of *Pleodorina illinoisensis* (Merton, 1908, p. 470, Text-figs. 1 and 2). He states that the two original cilia of the mother-cell persist and are found attached to one of the cells of the developing plakea. He also states that the developing plakea is kept fixed in a definite position inside the old mother-wall by means of the two old cilia. The colony is able to move inside the vesicle, "Brut-hole", only after their own cilia are developed. In Hartmann's figures of the inversion of the colony in *E. elegans*, the two cilia of the original mother-cell are shown as persisting on the original mother-wall, but they are not shown as being connected with any of the cells of the developing plakea (Hartmann, 1924, Fig. A, p. 379). There is also no reference to such a connection in the text of his paper. Iyengar\* found in *E. elegans* that the two original cilia are connected for quite a long time with one of the peripheral plakeal cells. He very often found

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\*From unpublished notes and figures kindly furnished by Prof. Iyengar.

that one of the mother cilia was connected with a peripheral cell of the plakea, while the other was connected with another cell which was situated at the opposite corner of the plakea (Text-figs. 16, 17). This is evidently according to him, brought about by the original protoplast dividing between the two ciliary portions. When the daughter colony is fully formed and is showing vibratile movements within the mother-cell vesicle, the two cilia can be very well seen attached to the wall of the mother-cell, but the inner connections of these to one of the cells of the daughter colony are no longer seen.

The eye-spot also remains in one of the cells. The eye-spot and the cilia of the mother-cell are on quite different cells (Text-figs. 13-15). Merton (1908, p. 469) has observed both on the same cell. The eye-spot remains bright for a time, but after the 16- and 32-celled stages, it is difficult to observe it.

After inversion, the daughter colonies remain moving inside the vesicle of their mother-cells for some time. The vesicles of the daughter-coenobia disappear soon after. The daughter colonies finally escape outside by the gelatinisation of the wall of the mother coenobium. Only in one case were the daughter colonies observed to escape singly one after the other from an opening at the posterior pole of a mother-coenobium. It was not clear whether this was a natural method or due to the pressure of the cover glass on the colony.

In the young colony just liberated the cells are very close together with hardly any space in the centre. The central space becomes noticeable only after the cells begin to enlarge and develop their mucilaginous wall layers. The colonies are at first oval or globose and become egg-shaped when they reach their adult condition. At first all the cells are of the same size and increase in size equally for a time. The cells of the first tier stop their growth and a little later the cells of the second tier also stop their growth. But the cells of the hinder tiers continue to grow until they all reach a fairly large size. In a young colony that has just escaped from the mother-coenobium all the cells have a single pyrenoid each. As the cells increase in size, the number of pyrenoids also increases. The pyrenoid formation stops after the cell reaches its full size. In the case of the cells of the first two tiers, since they stop their growth very soon, the increase in the number of the pyrenoids is only very small, *i.e.*, about 2-4. In the case of the cells of the other tiers, since the increase in cell size goes on much longer, the increase in the number of pyrenoids also goes on increasing upto about 8-13. Iyengar (1933, p. 335) has noted such an increase in the number of pyrenoids in *E. illinoisensis* and *E. indica*.

TABLE I

*Behaviour of the cells of the different tiers in the  
dividing colonies*

*Specimen I*

1st tier	4 undivided cells
2nd tier	8 undivided cells
3rd tier	9 undivided cells, 3 sperm bundles
4th tier	2 undivided cells, 10 sperm bundles
5th tier	1 undivided cell, 7 in plakea stages, 4 sperm bundles
6th tier	1 undivided cell, 1 in plakea stage, 10 sperm bundles
7th tier	4 sperm bundles

*Specimen II*

1st tier	4 undivided cells
2nd tier	4 undivided cells, 4 in plakea stages
3rd tier	2 undivided cells, 10 daughter colonies
4th tier	12 daughter colonies*
5th tier	12 daughter colonies
6th tier	12 daughter colonies
7th tier	4 daughter colonies

*Specimen III*

1st tier	4 undivided cells
2nd tier	8 undivided cells
3rd tier	7 undivided cells, 2 daughter colonies, 3 sperm bundles
4th tier	2 undivided cells, 2 daughter colonies, 8 sperm bundles
5th tier	3 undivided cells, 9 sperm bundles
6th tier	12 sperm bundles
7th tier	4 sperm bundles

*Specimen IV*

1st tier	4 undivided cells
2nd tier	5 undivided cells, 2 in plakea, 1 small daughter colony
3rd tier	12 daughter colonies
4th tier	12 daughter colonies
5th tier	12 daughter colonies
6th tier	12 daughter colonies
7th tier	4 daughter colonies

*Specimen V*

1st tier	4 undivided
2nd tier	8 undivided
3rd tier	10 daughter colonies, 2 sperm bundles
4th tier	12 sperm bundles
5th tier	12 sperm bundles
6th tier	12 sperm bundles
7th tier	4 sperm bundles



*Specimen VI*

1st tier	4 undivided
2nd tier	8 undivided
3rd tier	12 sperm bundles
4th tier	12 sperm bundles
5th tier	12 sperm bundles
6th tier	12 sperm bundles
7th tier	4 sperm bundles

*Specimen VII*

1st tier	4 undivided
2nd tier	7 undivided, 1 sperm bundle (small)
3rd tier	4 undivided, 8 sperm bundles
4th tier	1 undivided, 11 sperm bundles
5th tier	12 sperm bundles
6th tier	12 sperm bundles
7th tier	1 daughter colony, 3 sperm bundles

*Specimen VIII*

1st tier	4 undivided
2nd tier	8 undivided
3rd tier	1 undivided, 11 daughter colonies
4th tier	7 undivided, 5 daughter colonies
5th tier	4 undivided, 8 daughter colonies
6th tier	2 undivided, 10 daughter colonies
7th tier	4 daughter colonies

The cells of the front two tiers are generally somatic, but very occasionally the cells of the second tier show a tendency to divide (see Table I). Specimen II shows 4 undivided cells while the other 4 cells of the second tier are in various plakeal stages. Specimen IV has 5 undivided cells in the second tier, while of the remaining three two are in plakeal stages and the third has already reached the daughter colony stage. In Specimen VII, 7 cells of the second tier are somatic while one has formed a small sperm bundle. It may be seen from these cases that the small anterior cells, though somatic in general, occasionally show a tendency to divide. Iyengar (1933, pp. 337-39) found the four front cells of *E. illinoisensis* showing division and occasionally even forming sperm bundles. Grove (1916, p. 176) also found the front cells showing divisions.

Often the anterior portion of a colony with only the two front tiers of somatic cells were found in the material. These are evidently portions of dividing mother colonies which were left behind intact after the escape of the daughter colonies from the posterior portion of the colony. The cells of the second tier, in these were often found to show some division stages for a long time. These anterior portions were kept under observation for a long time. They did not show any further division stages, but degenerated and died out.

## SOMATIC MITOSIS

*The resting nucleus.*—The nucleus is situated in the anterior portion inside the hollow of the cup-shaped chloroplast. It is

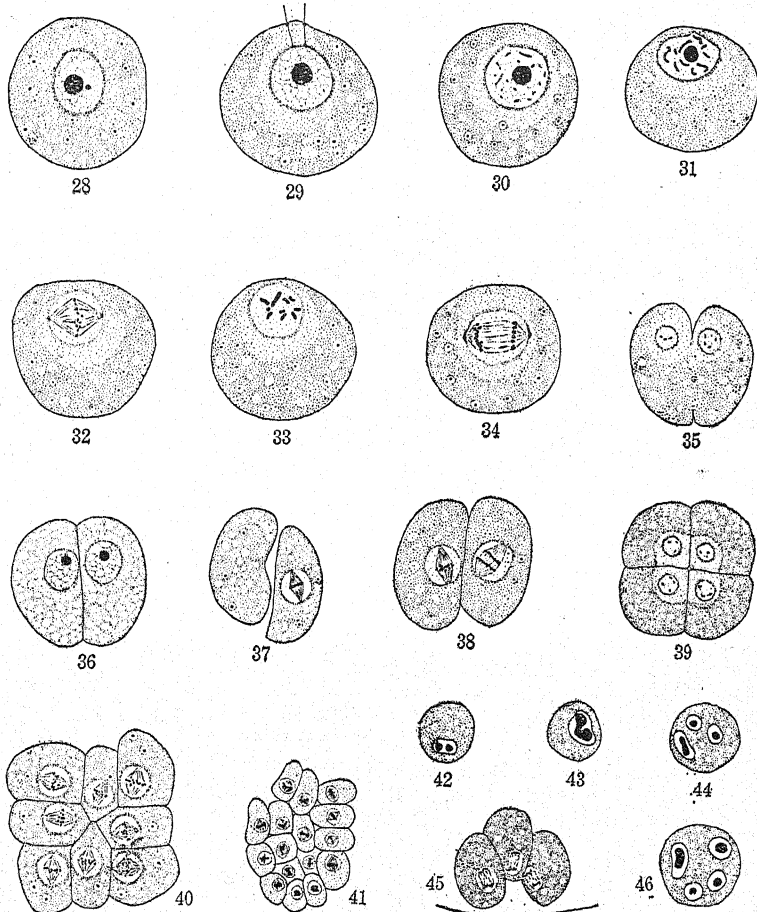


spherical but often elongated and contains a large deeply staining nucleolus. The outer nucleus appears clear, but in very favourable preparations a faintly stained reticulum could be seen in it. In addition to the nucleolus a small darkly staining granule could be seen (Text-fig. 28; Pl. III, Fig. 10) quite similar to what Hartmann (1921, p. 230) observed in *E. elegans*. Hartmann found that this body in *E. elegans* divides into two and the spindle is formed between them. Later on the two bodies are found at the two poles of the spindle at metaphase. From this behaviour, he thought that this dark body should be considered as a centriole. In the present alga the division of this dark body into two and the formation of the spindle between them could not be seen. But, during metaphase, two dark bodies are seen, one at each pole of the spindle (Text-figs. 32, 37; Pl. III, Fig. 16), not quite unlike the centrosomes figured by Hartmann (1921, Taf. 2, Fig. 14). It is just possible that the two bodies found at the poles of the spindle during metaphase were derived from the single dark body found in the resting nucleus.

The normal resting nucleus in a fully developed cell is about  $4-5\ \mu$  in diameter, but at the beginning of the prophase, it becomes slightly enlarged and measures about  $6-7\ \mu$ . The reticulum in the outer nucleus becomes somewhat more deeply stained and the chromatin material can be seen as many tiny dark bodies imbedded in it (Text-fig. 30; Pl. III, Fig. 11). A little later, long thread-like structures could be observed in the outer nucleus. These thread-like structures condense still further and form the chromosomes. At this stage the nucleolus is still quite intact and darkly stained (Text-fig. 31; Pl. III, Fig. 12). The chromosomes are slightly longer than those in the metaphase stage. A count made in this prophase stage shows ten chromosomes.

The spindle is intranuclear and is first seen in metaphase. At about this time the nucleolus disappears and a little later the nuclear membrane also disappears. The chromosomes are short and rod-shaped and lie in a plate at the equatorial region of the spindle (Text-fig. 32). The number of chromosomes counted from the polar view is 10 and this number agrees with the number obtained from a count in the late prophase stage also (Text-fig. 33; Pl. III, Fig. 13). Ten chromosomes have been recorded in *E. elegans* by Hartmann (1921), and in *E. illinoisensis* by Hovasse (1937). But Merton (1908) recorded twelve chromosomes in *Pleodorina illinoisensis* (*E. illinoisensis*).

In the anaphase, the daughter chromosomes move towards the poles (Text-fig. 34; Pl. III, Fig. 15). A little accumulation of cytoplasm is seen at the ends of the spindle poles. It is very much like that figured by Hovasse (1937, Pl. VIII, Fig. 24) in *E. illinoisensis*. At the telophase each group of chromosomes re-organises into a daughter nucleus with a distinct nucleolus and a faint reticulum round it. In some of the preparations, at the two-celled stage, the nuclei were observed to contain more than one (usually two or three), darkly staining bodies within them (Text-fig. 35).



Text-Figs. 28-46. *Eudorina indica* Iyengar. Fig. 28. A cell with a resting nucleus inside which is a nucleolus and a centriole ( $\times 1205$ ). Fig. 29. Cell showing the nucleus, two rhizoplasts and the basal granule of the cilia ( $\times 1205$ ). Fig. 30. Early prophase showing chromatic concentration ( $\times 1205$ ). Fig. 31. Late prophase showing 10 chromosomes in the outer nucleus ( $\times 1205$ ). Fig. 32. Metaphase and spindle showing centrosome-like bodies at the poles ( $\times 1205$ ). Fig. 33. Polar view of metaphase showing 10 chromosomes ( $\times 1205$ ). Fig. 34. Anaphase ( $\times 1205$ ). Fig. 35. Cleavage of the protoplast after nuclear division ( $\times 1205$ ). Fig. 36. Two-celled stage ( $\times 1205$ ). Fig. 37. Two-celled stage with the nucleus of one of the cells at metaphase stage. Centrosome-like bodies are seen at the poles of the spindle ( $\times 1205$ ). Fig. 38. Two-celled stage with the nuclei of the cells in early anaphase stages ( $\times 1205$ ). Fig. 39. Four-celled plakeas ( $\times 1205$ ). Figs. 40-41. 8- and 16-celled plakeas with all the nuclei in metaphase stage. Fig. 40 ( $\times 1205$ ). Fig. 41 ( $\times 956$ ). Fig. 42. Cell with a pyrenoid in which the pyrenocrystal has already divided into two inside the still undivided starch sheath ( $\times 440$ ). Fig. 43. Cell with the pyrenocrystal of a pyrenoid constricted ( $\times 440$ ). Fig. 44. Four-celled plakea in section showing three cells with their nuclei in late anaphase stages ( $\times 956$ ). Figs. 45-46. Cells with elongated pyrenoid ( $\times 440$ ).

Finally when the two cells are fully organised only one dark body, the nucleolus, is seen within the nucleus.

Cell-division takes place immediately after the nuclear division is complete. A fine cleavage-furrow is formed at either end of the cell in a longitudinal plane (Text-fig. 35). This furrow finally divides the cell into two. In the subsequent divisions the mitosis is quite similar, only the nuclei get smaller as the divisions proceed.

#### PYRENOIDS AND THEIR BEHAVIOUR

Whether the pyrenoids arise *de novo* or through the division of a pre-existing one is still a disputed point. Carter (1926, pp. 669-70) has given a brief account of the observations of the earlier workers. Goroschankin (1875) reports that pyrenoids disappear during division in *E. elegans*. Merton (1908, p. 460) thinks that *de novo* formation is possible because of the diversity in the size of the pyrenoids. Grove (1916) found no visible pyrenoids in the young daughter colonies and concluded that they must be formed *de novo*. Hartmann (1919) found that the pyrenoids in *Chlorogonium elongatum* gradually dissolved at the first nuclear division, leaving for a short time several darkly staining portions. They disappear in early prophase and reappear in young daughter cells. Hartmann (1921) found that in one strain of *E. elegans* only one pyrenoid was found in each cell. This pyrenoid divided regularly along with the division of the chromatophore. In another strain of the alga, several pyrenoids were found in each cell. In this he observed an increase in the number of pyrenoids upto 32, and each of the daughter cells eventually possessed one pyrenoid. Zimmermann (1921, p. 264) at first considered that the pyrenoids arise *de novo* in *Volvox* but later on he revised his opinion after a study of the living material and came to the conclusion that they arise through division (Zimmermann, 1925). Geitler (1926, p. 142) states that pyrenoids generally appear to dissolve at the beginning of swarmer-formation. Carter (1926, p. 670) while working on *Ulva lactuca*, recognises two kinds of processes, (1) a solution process and (2) a fragmentation process with the dispersion of the fragments throughout the chloroplast. She considered that both these processes might go on at the same time in all the cells. Pocock (1933, p. 552) considers that there is enough evidence in microtome preparations as well as in living developing daughters of *Volvox* to show that pyrenoids divide.

In the present alga, the author found plenty of evidence for the division of the pyrenoid in the microtome preparations. During division, the pyrenoid as a whole including both the starch sheath and the pyreno-crystal lengthens and then the pyreno-crystal divides into two while still surrounded by the starch sheath. Later on, the starch sheath also divides and the entire structure divides into two halves by constriction (Text-figs. 42-44 and 46). In the dividing cells the number of pyrenoids is larger, but the sizes of the pyrenoids gradually becomes smaller and smaller. These pyrenoids

become so small that at first sight the dividing cell appears as if devoid of any pyrenoids, but a careful examination of the sections shows a large number of minute pyrenoids which at first sight appear as very small darkly stained granules. In the cell of the young daughter colony, it is very difficult to make out the pyrenoid, but it becomes visible when the colonies grow a little larger. At first only one pyrenoid is found in each cell of the very young daughter colony.

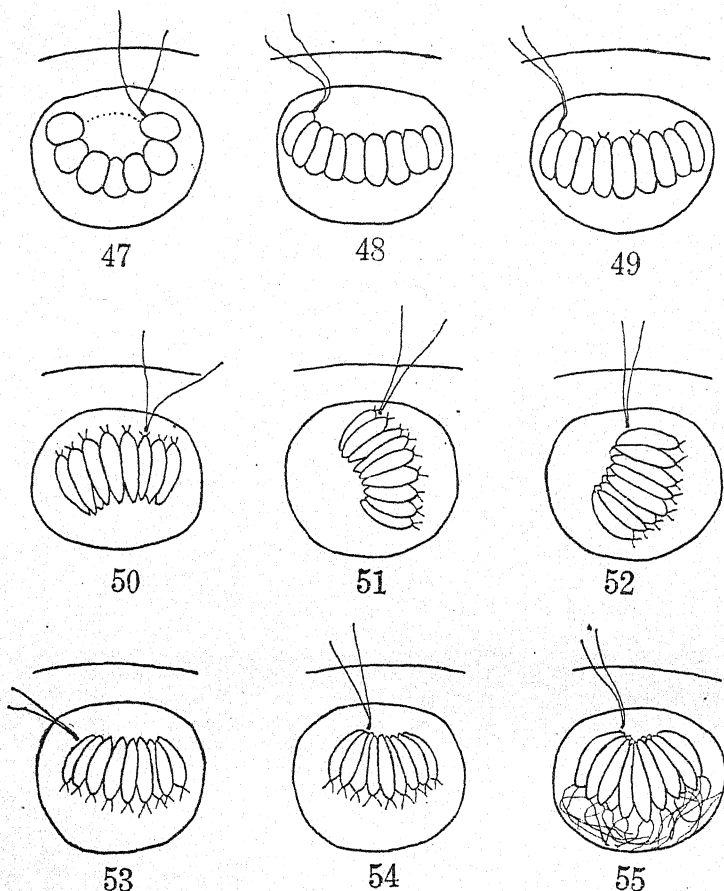
#### SEXUAL REPRODUCTION

The colonies are asexual or sexual and dioecious. In the sexual colonies, as in the asexual ones, the front 12 cells are somatic, while the remaining cells form either antheridia or oogonia. In a number of sexual colonies (both male and female) some of the reproductive cells formed daughter coenobia instead of antheridia or oogonia (Table I, p. 126).

*Development of the antheridia.*—The antheridia contain in some cases 32 and in other cases 64 sperm-cells. The antheridial initial divides by a series of divisions and forms a plakea. The details of cell-division are quite similar to those of the daughter colonies. The plakea throughout its development is enclosed within the vesicle formed by the gelatinised wall of the antheridial mother-cell. The two cilia of the parent cell remain attached to one of the plakeal cells (Text-fig. 47). Occasionally the two mother cilia are attached to two separate cells of the developing plakea.

*Inversion of the sperm bundle.*—As already mentioned inversion of the daughter colony has been recorded in *Volvox* by several workers (Powers, 1908; Kuschakewitsch, 1923; Zimmermann, 1925; and Pocock, 1933). But Pocock (1933, p. 581) found that inversion takes place during the formation of the sperm bundles also in *Volvox*. In *Eudorina elegans* Hartmann (1924) found that inversion takes place during the formation of daughter colonies. But he does not say anything about the occurrence of inversion during the formation of the sperm bundles in the alga. Pascher (1927, p. 437) states that in *Eudorina* the plate of cells which are to form the sperm bundles does not curve and show any inversion. Fritsch (1935, p. 115) states that the sperm bundles of *Eudorina* are formed by successive division following the same sequence as in ordinary vegetative multiplication, but usually culminating in the production of 64 units and unaccompanied by any appreciable incurling of the resulting plate.

Professor Iyengar and the author found that in *Eudorina elegans* inversion takes place not only in the daughter colonies but also in the sperm bundles. Unfortunately the author missed recording the process in the living material of *E. indica*, but a careful examination of the preserved material of *E. indica* shows clear indications that inversion takes place during the formation of the sperm bundles in this alga also. The antheridial plakea in *E. indica* is at first flat, but, in the later stages, the plakea becomes definitely cup-shaped (Text-fig. 47; Pl. II, Fig. 9). The concave side of the cup-shaped



Text-Figs. 47-55. *Eudorina indica* Iyengar. Stages of inversion during the development of sperm bundle. Fig. 47. Plakea, cup-shaped, with its concave side directed towards the outside of the colony at the beginning of the inversion ( $\times 1150$ ). Fig. 48. Cup-shaped plakea flattening during inversion ( $\times 1150$ ). Fig. 49. Plakea getting more flattened; cilia at the outer end of the cells ( $\times 1150$ ). Fig. 50. The plakea getting curved outwards; orientation of the whole plakea just beginning ( $\times 1150$ ). Figs. 51-54. Further stages in the orientation of the sperm bundle on its own axis ( $\times 1150$ ). Fig. 55. Fully formed sperm bundle ( $\times 1150$ ). Mother-cell cilia attached to one of the plakeal cells in Figs. 47-49. Attachment of the mother cilia with the plakeal cell broken off at the beginning of the orientation in Fig. 50. Mother cilia not connected with the plakeal cell, though remaining very close to the plakea in Figs. 51-55.

plakea is directed to the outside of the mother-colony. At this stage inversion commences, and the cup-shaped plakea begins to curve in the opposite direction. As a result of this inversion, the cup-shaped plakea becomes flat once more. About this stage the cells of the plakea become slightly elongated at right angles to the

plane of the plakea, and at the same time the beginnings of the cilia could be just seen at the ends of the plakeal cells directed to the outside of the colony. The two cilia of the mother-cell of the plakea could be still seen attached to one of the cells of the plakea (Text-fig. 49). As the inversion continues the plakea becomes slightly curved outside, *i.e.*, its convex side becomes directed towards the outside of the colony (Text-fig. 50). The individual cells of the plakea then become slightly broader towards the outer (ciliary) end and narrower towards the inner end. After this the whole plakea shows a peculiar orientation movement. It turns gradually on its own axis as it were to about  $180^\circ$ . Before the commencement of this orientation movement the ciliary ends of the cells of the plakea are all pointing towards the outside of the colony. But after the completion of this movement, the ciliary ends of the cells are all pointing towards the inside of the colony (Text-fig. 54). During this process of orientation, the attachment of the two cilia of the old mother-cell with the one or two plakeal cells becomes broken off at the base of the cilia. At first sight the two cilia appear as if they are attached to the orienting bundle, but a careful examination shows they are not actually connected with it but are merely remaining very close to it. Merton (1908, Pl. XXVII, Fig. 6) has figured a bundle in this condition. He has however shown the two mother-cilia attached to one of the cells of the bundle. But the present study shows that the connection between the mother cilia and the cell of the antheridial bundle is only apparent and not real, since this connection becomes broken off at the beginning of the orientation movement of the plakea.

By the time the orientation is completed, the cilia of the antheridial cells are fully developed and the bundle begins to show oscillatory movements inside the vesicle. Soon the vesicle disappears and the bundles escape outside through the gelatinisation of the matrix of the mother colony.

So here we see that a definite inversion takes place during the formation of the sperm bundles. The inversion process is quite similar to that of the daughter colony upto the stage when the cup-shaped plakea becomes flattened once again. After this stage, in case of the daughter colony, the inversion process continues until the plakea becomes completely curved backwards and closes up finally into a more or less spherical colony with the cells disposed all-round and with their ciliary ends directed outside. But, in case of the sperm bundle, the inversion process continues only for a shorter duration, *i.e.*, upto the stage when the plakea becomes slightly curved backwards. The plakea here does not close up completely into spherical structure as in the case of the daughter colony, but becomes only a somewhat hemispherical structure. And, since the cells of the plakea ultimately become broader near their outer cilia bearing ends and narrower towards the opposite ends, the concave (inner) side of the plakea becomes very much reduced in size, while its convex (outer) side becomes very much

broadened. When the antheridial bundle is fully formed the concave side appears merely as a small depression at its posterior end (Text-fig. 55).

The peculiar orientation movement of the whole plakea through  $180^\circ$  has nothing to do with the process of inversion, though the final stages of the inversion may be going on while the plakea is slowly getting oriented. Why this orientation takes place is not clear. But every bundle becomes oriented in this way before the cilia are fully developed and the whole bundle begins to show any active movement by means of its fully formed cilia.

The bundles after escaping swim freely in the water for a long time, all the while rotating on their own axes. Several bundles could be seen hovering a long time round a female colony and finally break up into the individual sperms. The sperms when free reach the oogonia and move very actively round the eggs.

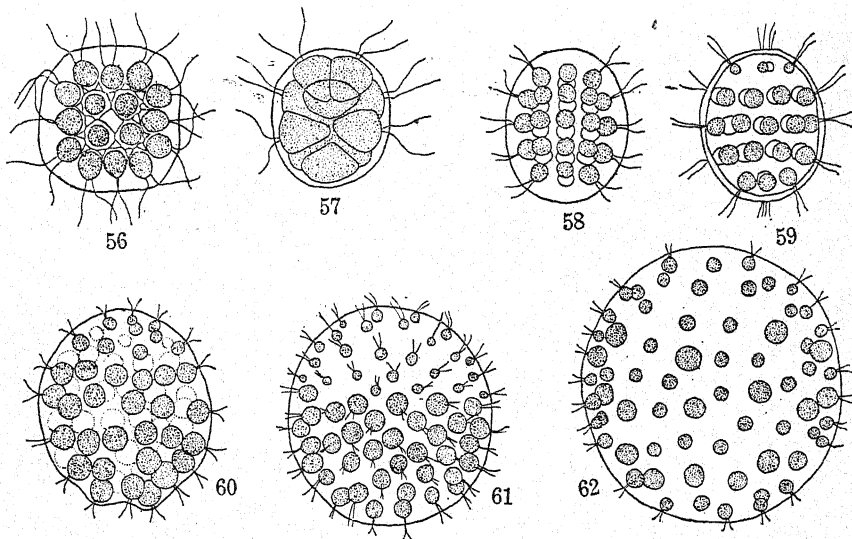
The sperm is elongated and spindle-shaped and is broader in front and very narrowly elongated behind and measures  $2-4\ \mu$  wide at its broadest part and  $12-14\ \mu$  long. Its two cilia are about  $1\frac{1}{2}$  times as long as its body. They are attached not apically but a little below the apex behind a small beak-like anterior end (Text-fig. 8; Pl. II, Fig. 8). The chloroplast of the spermatozoids is faintly green. It contains a single small pyrenoid. In its anterior hyaline part are seen a single nucleus and an eye-spot. The nucleus is seen only in stained preparations.

*Development of oogonia.*—Mature female coenobia can be well distinguished from the male or asexual colonies by their rich deep green colour. The fully developed antheridial initial of the male colony is not so green as the fully developed oogonial initial. The gelatinous matrix of the fully developed female colony gradually breaks down and becomes softened. The thick outer gelatinous layer of the walls of the egg cells also is soon lost, but the cells are still surrounded by their inner thin firm membrane. After a time even this is lost by gelatinisation and the cells lie loose within the soft gelatinous matrix of the colony. The cells still retain their cilia, become slightly pear-shaped and show a slight movement inside the loosened matrix (Text-fig. 9; Pl. II, Fig. 5). The movement is more or less oscillatory and confined to a very limited space. Number of sperm cells come and swarm round the egg cells. They are often seen crowding over them but after a time leave off and swim away without effecting fusion. Single egg cells were often found moving slightly away from the general matrix of the coenobium and were then surrounded by the male cells, but even here no fusion could be observed. Actual entry of the sperms into the egg was not seen even in a single case, nor were four ciliated oospores observed, even though a number of egg-cells surrounded by sperm cells were observed continuously for a long time. Some time after the swarming of the male cells the egg-cells were found to have lost their cilia and become round once again and to have formed walls round themselves.



Towards the end of the season the colonies float up in large numbers forming a greenish frothy scum at the surface. The gelatinous envelope of most of the coenobia loses its firm smooth outline and becomes somewhat loose and sticky and soon gets covered by adhering silt particles (Pl. I, Fig. 6). Plenty of sperm-cells are found round the cells inside these colonies showing clearly that these are female colonies. Whether these thick-walled cells represent fertilised egg-cells or merely vegetative cells covered with thick walls as a preparation for perennation could not be made out. These cells show a certain amount of resemblance to the aplanospores of *E. elegans* as figured by Pascher (1927, Fig. 400), but the walls of the resting cells in the present alga are not crenate as figured by Pascher in *E. elegans*, but are quite smooth. As the pool dried up, the frothy floating scum containing countless gelatinised colonies and also plenty of vegetative colonies dried up completely.

Iyengar (1933, p. 311), when he first described this species, found only one colony forming daughter colonies in his material. From the behaviour of this dividing colony and also from the fact that the cells of the two front tiers were smaller than the rest, he suggested that the front two tiers are somatic and that the remainder were reproductive. The present investigation on the living alga fully confirms this suggestion. In the case of *Gonium*



Text-Figs. 56-62. Some colonial Volvocales. Fig. 56. *Gonium pectorale* Müll. (after Hartmann). Fig. 57. *Pandorina morum* (Müll) Bory (after Pringsheim). Fig. 58. *Eudorina elegans* Ehr. (after Hartmann). Fig. 59. *E. illinoisensis* Pascher (*Pleodorina illinoisensis* Kofoid) (after Kofoid). Fig. 60. *E. indica* Iyengar (after Iyengar). Fig. 61. *Pleodorina californica* Shaw (after Chatton). Fig. 62. *P. sphaerica* Iyengar (after Iyengar).



*pectoralis* Müll, *Pandorina morum* (Müll) Bory and *E. elegans* Ehrenb., all the cells are reproductive though the cells of the front tier in *Eudorina elegans* showed a slight tendency to become smaller and to behave differently from the remaining cells (Iyengar, 1933, p. 333). In the case of *E. illinoisensis* (Kcfoid) Pascher the front four cells have become somatic though still showing a tendency to divide frequently. In the case of *E. indica* Iyengar the cells of the front two tiers have become somatic, though they may show a tendency to divide. It may however be mentioned that this tendency to divide is seen more in the cells of the second tier than in those of the first. In *Pleodorina californica* Shaw, the process of sterilisation has gone still further, the cells of the anterior half of the colony becoming somatic and the remainder being reproductive in nature (Fritsch, 1935, p. 115). In *P. sphaerica* Iyengar (Iyengar, 1933, p. 343) the sterilisation has gone still further, a number of cells in the posterior portion of the colony also becoming somatic. This is a step leading definitely towards an organisation found in *Volvox* where an extremely large number of cells in the posterior portion of the colony has become somatic, only a few cells retaining their gonidial nature. Iyengar from a study of his preserved material of this alga suggested that *E. indica* stands between *E. illinoisensis* and *Pleodorina californica*. The present study of the living material of the alga fully confirms this suggestion.

#### SUMMARY

An account of the life-history of *Eudorina indica* Iyengar as studied from the living material is given in the paper.

The colony contains 64 cells in seven tiers of 4, 8, 12, 12, 12, 12 and 4 cells respectively. The cells of the first two tiers are smaller than those of the remaining tiers. They are somatic and serve to direct the colony. The cells of the remaining tiers are reproductive in nature. The colonies are either asexual or sexual. The sexual colonies are dioecious.

The reproductive stages of the alga commence only after the alga has remained in a vegetative condition for a very long time.

The stages of development of the daughter colony are quite similar to those of *E. elegans*. Inversion takes place during the formation of the daughter colony.

Inversion takes place during the formation of the antheridial bundle also. As the inversion is nearing completion, the whole bundle becomes oriented on its own axis to about 180°.

The spermatozooids when free, swim towards the female colonies and swarm round the egg cells. But no case of actual fusion was observed even though numerous female colonies in which the spermatozooids were actively swarming round the eggs were kept under continuous observation for quite a long time.

Somatic mitosis was studied in detail. The number of chromosomes is ten. In the resting nucleus, close to the nucleolus, a small

darkly staining body was observed similar to the one described by Hartmann in *E. elegans*.

The pyrenoids in the present alga appear to arise through the division of pre-existing pyrenoids and not *de novo*.

This alga is more advanced than *E. illinoisensis* and stands between it and *Pleodorina californica*.

The author wishes to express his great indebtedness to Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., for his constant guidance and help during the course of this investigation and in the preparation of this paper. His sincere thanks are also due to the authorities of the University of Madras for the award of a research scholarship during the tenure of which the present investigation was carried out.

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## EXPLANATION OF PLATES

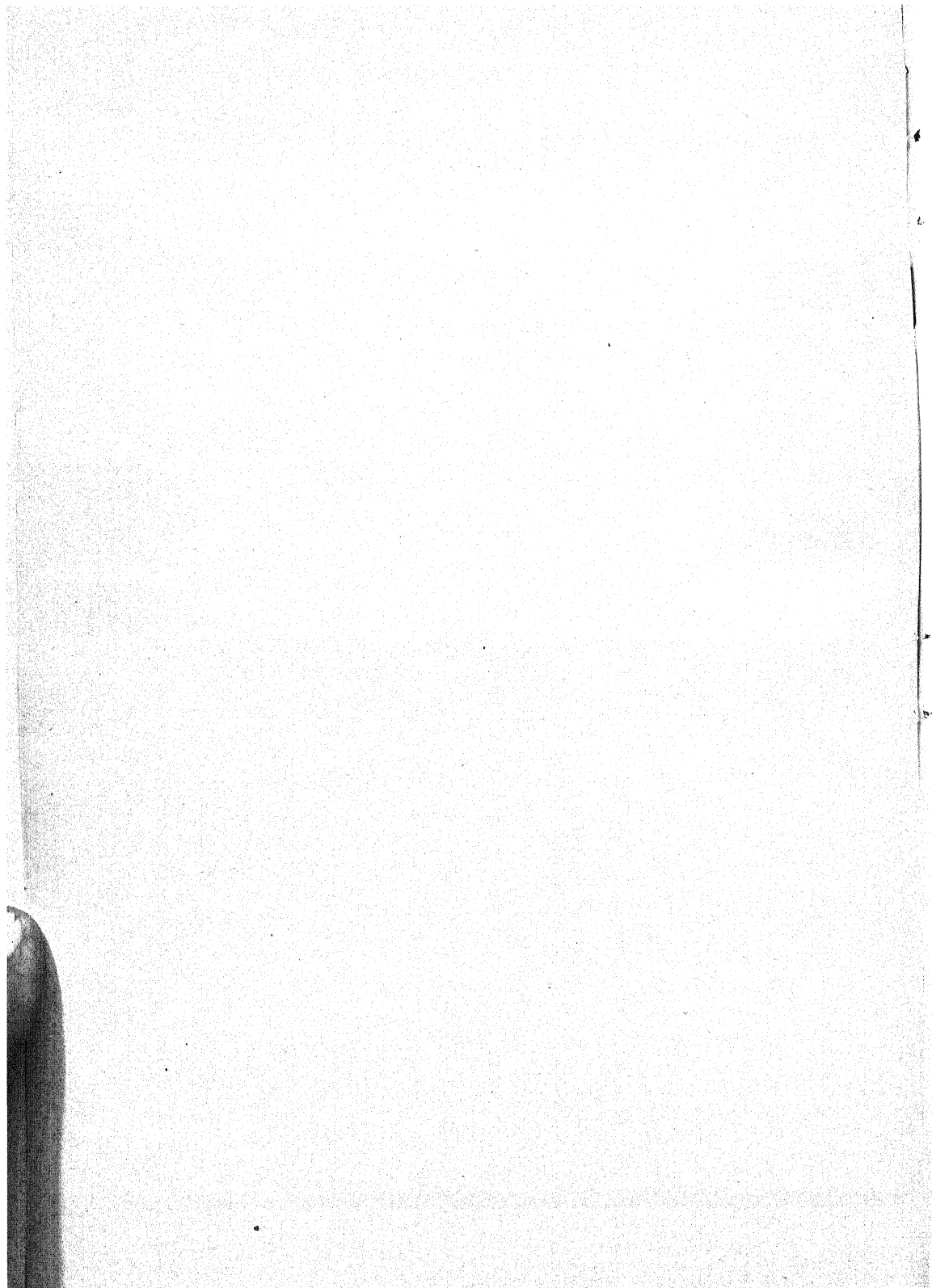
*Eudorina indica* Iyengar

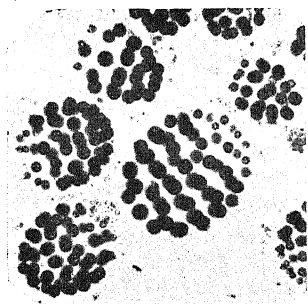
## PLATE II

- FIG. 1. Vegetative colonies both 32- and 64-celled. ( $\times 62$ .)  
FIG. 2. A colony with fully developed cells about to divide. ( $\times 235$ .)  
FIG. 3. A dividing colony. ( $\times 132$ .)  
FIG. 4. A portion of a dividing colony. Note the mother-cell cilia attached to a few developing daughter coenobia. ( $\times 276$ .)  
FIG. 5. A naked egg cell with its two cilia still attached. ( $\times 494$ .)  
FIG. 6. A group of colonies about to hibernate (?). Note the loosened gelatinous matrix covered by silt particles. ( $\times 35$ .)  
FIG. 7. Plaque of the developing sperm bundle flattened. ( $\times 350$ .)  
FIG. 8. Spermatozooids swarming round the egg cell. ( $\times 494$ .)  
FIG. 9. A portion of a dividing colony; note the cup-shaped plaque of the developing sperm bundle just before inversion. ( $\times 350$ .)

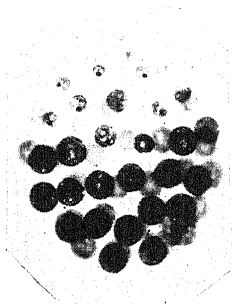
## PLATE III

- FIG. 10. A cell showing resting nucleus with the nucleolus and a centriole. ( $\times 2018$ .)  
FIG. 11. Early prophase. ( $\times 1835$ .)  
FIG. 12. Late prophase showing chromosomes and the persisting nucleolus. ( $\times 1835$ .)  
FIG. 13. Polar view of metaphase. ( $\times 1835$ .)  
FIG. 14. Metaphase; note the nucleolus has disappeared. ( $\times 880$ .)  
FIG. 15. Anaphase. ( $\times 1835$ .)  
FIG. 16. 2-celled plaque with the nucleus of one of the cells in metaphase. ( $\times 1835$ .)  
FIG. 17. An 8-celled plaque with all the nuclei in metaphase. ( $\times 1282$ .)  
FIG. 18. A 16-celled plaque showing all the nuclei in metaphase. ( $\times 853$ .)

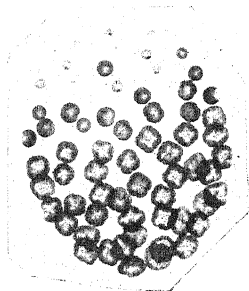




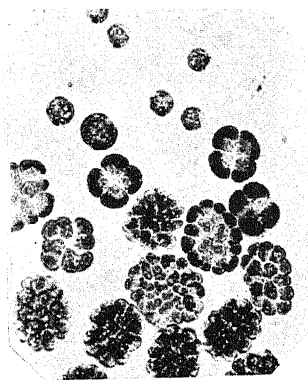
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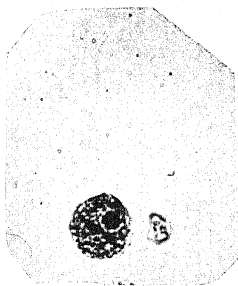
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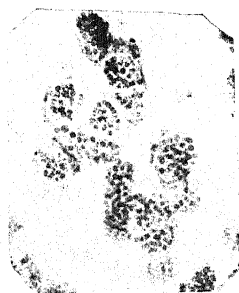
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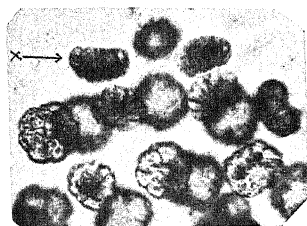
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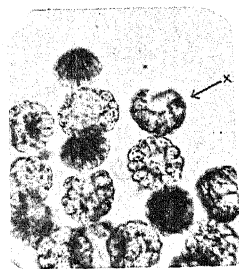
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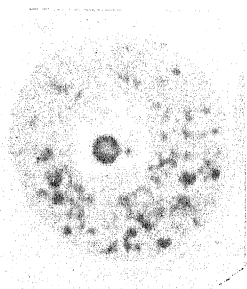


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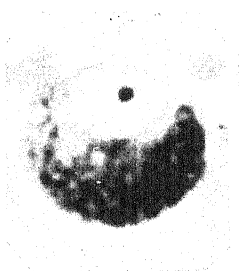


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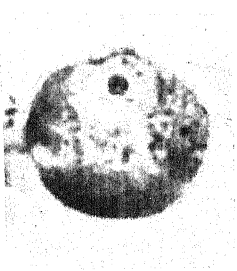




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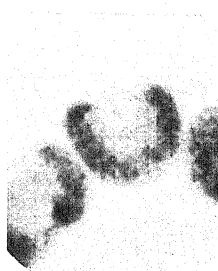
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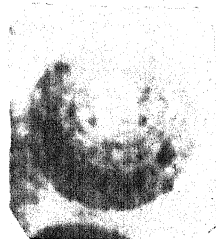
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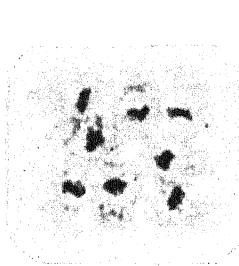
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SYSTEMATIC POSITION OF A LITTLE KNOWN  
FLOWERING PLANT FROM SOUTH BURMA

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Received for publication on July 20, 1940

EXAMINATION of the original Wallich's specimens proves that there exists considerable confusion in some of them as regards the correct systematic position. Such confusion in many cases is evidently due either to incomplete materials or hurried determination. Two Wallich's specimens collected in 1827 from South Burma illustrate that they are two distinct plants belonging to two different families although the sheets bear the same name (*Microtropis longifolia* Wall.) and the same Wallich's number 4339. Prof. E. D. Merrill of the Harvard University, U.S.A., while revising the genus *Microtropis* was furnished with a sketch of Wallich's 4339, collected from Tavoy on the 2nd. October 1827, together with a note from Mr. V. Narayanaswami who rightly considered these two Wallichian sheets as two separate species. His decision was based on the character of the fruit, only one of which was found along with the Wallich's sheet mentioned above. Wallich's sheet No. 4339, collected from Tavoy on the 4th. November 1827, is a *Salacia*, and it is entirely different from the other specimen, a *Microtropis*, collected on the 2nd October from Tavoy bearing the same number.

Prof. Merrill consulted the Kew authorities who declared that all the Wallich's sheets No. 4339 were nothing but *Salacia flavesces*. On account of the difference of opinion between Kew and Calcutta and as far as could be made out from the sketch sent to Dr. Merrill, Dr. Merrill could not definitely fix the correct systematic position of the two sheets of Wallich differing from each other. Prof. Merrill finally referred the question to me in the following words :—

"I am still puzzled regarding *Microtropis longifolia* Wall., i.e., Wallich 4339 in the Calcutta Herbarium which we did not see but of which you courteously sent an excellent drawing. I am including it as a valid species, but Airy-Shaw wrote me from Kew that all the materials of Wallich 4339 at Kew actually represented *Salacia flavesces* Kurz. If you can find time to do so, cannot you critically re-examine Wallich 4339 in the Calcutta Herbarium, compare it with *Salacia flavesces* Kurz, and let me know what you think about it?; that is is it *Microtropis* or is it the *Salacia*? Superficially there is much resemblance between the two otherwise totally different species."

My careful scrutiny of the sheets of *Microtropis* with special reference to *M. longifolia* Wall. (No. 4339) reveals that the two Wallichian

sheets are undoubtedly separate species and belong to two different families. Wallich's sheet No. 4339, a leafy specimen collected from Tavoy on the 4th. November 1827, is a *Salacia* belonging to the family *Hippocrateaceae*, and the sheet No. 4339, a fruiting specimen collected from Tavoy on the 2nd. October 1827, is a *Microtropis* belonging to the family—*Celastraceae*. My definite decision is based not only on the fruiting and leafy specimens but also on two complete flowering specimens, one from the same locality as that of Wallich's and the other from the District Thaton, South Burma. These sheets were received in the Herbarium after a period of little over a century. The flowering specimen No. 6553 collected by Mg. Po Chin, on the 24th. March 1928, was correctly identified as *Microtropis longifolia* by Mr. V. Narayanaswami as it agrees with Wallich's 4339 of October 1827. In scrutinising these sheets Prof. Merrill, even without seeing the fruiting specimen of Wallich's type, considered it a new species and suggested a new name *M. pachyphylla* Merr. & F. But this specimen is a true *Microtropis longifolia*. This was already determined as such by Mr. Narayanaswami and confirmed by the writer. Prof. Merrill was definitely informed about the identity of these sheets in our notes and correspondence. It was expected that he will make necessary modifications in his monograph on the genus *Microtropis* taking *M. longifolia* as a genuine species and deleting or sinking in it his suggested name *M. pachyphylla*. The specimen No. 13105, collected from Tavoy by Maung Ba Pa on the 7th. March 1932 is also an exact match of the Wallich's fruiting specimen No. 4339 collected from Tavoy on the 2nd. October 1827 and marked by Wallich as *Microtropis* ? *longifolia* Wall.

The other Wallich's sheet No. 4339 is a leafy specimen collected from Tavoy on the 4th. November 1827. This plant matches with *Salacia flavescens* and should be taken as such. It is surmised that the controversy between Kew and Calcutta is due evidently to Kew Herbarium not possessing the sheet 4339, collected on the 2nd. October 1827. All the Wallich's sheets 4339 of the 4th. November and 4339  $\beta$  are true *Salacia flavescens*. Wallich sheets No. 4338 also are all different forms of *S. flavescens*. The sketches of the recent plant of *M. longifolia* and the photo of the type sheet will clearly show the generic differences. The striking distinctions lie in the stamens and the capsule.

#### Family—*Celastraceae*

Stamens 5, inserted on the disk or on the tube of corolla. Fruit a capsule, elliptic with persistent calyx and surmounted with a stout conical point, 2 valved, 1 celled, 1 seeded. . . *Microtropis*.

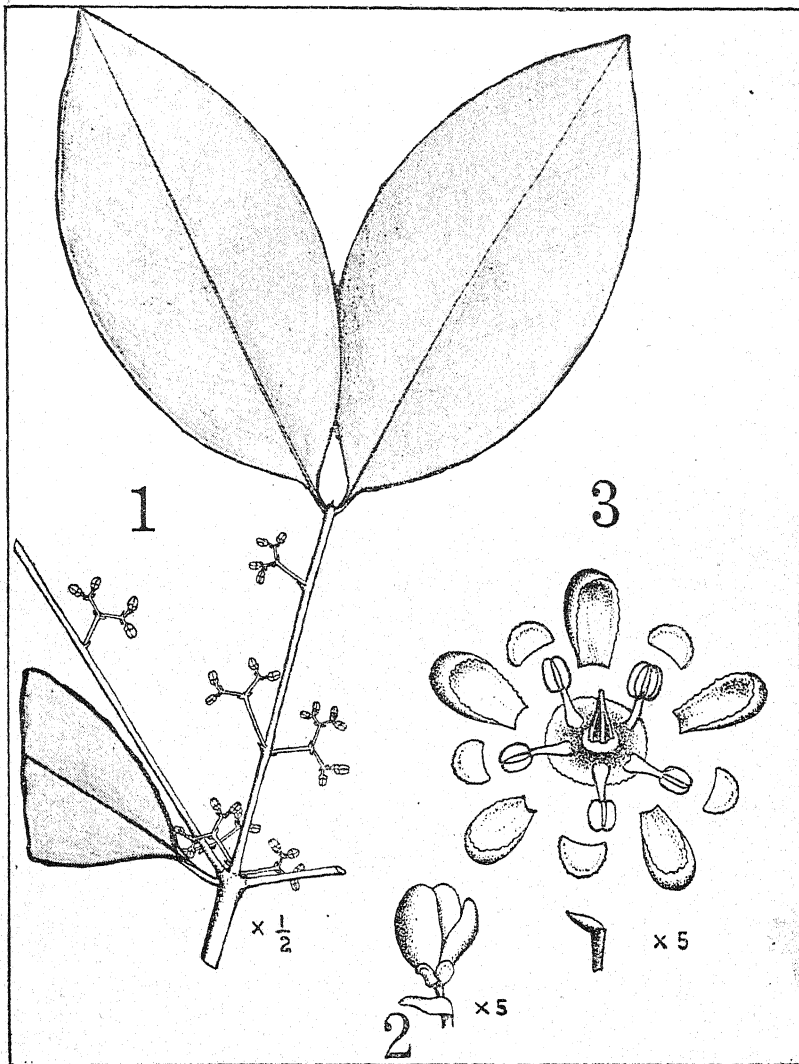
#### Family—*Hippocrateaceae*

Stamens 3 rarely 4, continuous with the disk, recurved. Fruit large of the size of a plum, baccate, fleshy or more or less woody. . . *Salacia*.

Up to 1873 Wallich's name on sheet No. 4339 (2nd. October 1827) remained *nomen nudum*. In 1873 S. Kurz in his Forest

Flora of Burma added an insufficient but technical description for the first time.

Under the description of *Salacia flavescens* Kurz., the name *M. longifolia* with the Wallich's No. 4339 was referred to by Lawson on page 625 of the "Flora of British India," Vol. I, 1875, as agreeing with *Salacia flavescens* in part. It has also been noted under *M. latifolia* (a South



*Microtropis longifolia* Wallich. Fig. 1. A flowering branch (reduced  $\frac{1}{2}$  Nat. Size) showing arrangements of leaves and inflorescence. Fig. 2. Magnified flower ( $\times 5$ ), and Fig. 3 showing dissection of floral parts ( $\times 5$ ).

Indian Species) in "Flora of British India," page 613, Vol. I, 1875, as a synonym in part; but Gamble in his "Flora of the Madras Presidency" treats *M. latifolia* as a separate species and does not mention about its any similarity to *M. longifolia* Wall. In fact comparison between *M. latifolia* and *M. longifolia* points out that the two plants are entirely different from each other. I am describing here the complete flowering and fruiting materials of *M. longifolia* Wall. I retain the Wallich's name as noted in his own handwriting on the sheet 4339 of October 1827. The name is valid as Kurz added a description of the plant, though very inadequate, in his "Forest Flora of Burma," Vol. I, page 250, 1877, and earlier (*Pro Parte*) in Latin published in the *Journal of the Asiatic Society of Bengal*, Vol. XLII, page 65, 1873.

*Microtropis longifolia* Wallich

*M. longifolia* Wallich affinis *M. filiformis* King, foliis acutis. Coriaceis, supra glabris, infra glauciscentis, nervis primariis lateralibus supra et infra inconspicuis, petioli late marginatis, inflorescentia sparse ramosa divaricata dichotoma, capsula oblongo-ellipsoidea, basi et apice leviter attenuata distincta.

A much branched small tree, about 2-3 m. tall; branchlets terete, glabrous, dark brown, sometimes almost black. *Leaves* exstipulate, shortly petioled, opposite, obovate, broadly elliptic, acute, cuneate at the base, entire, margin more or less revolute and somewhat cartilaginous, leathery, dark green and glabrous above, pale green and glaucous beneath, lateral nerves 3-5 pairs, indistinct on both the surfaces, midrib impressed on the upper surface, prominent on the lower surface; lamina 11-18 cm. long, 5-8 cm. broad; petioles 8-16 mm. long, brown, glabrous, channelled, distinctly marginate. *Inflorescence*, cymose, sparsely dichotomously branched, extra-axillary divaricate; peduncles 1.5-3.5 cm. long subtended by a pair of bracts at the point of divarication; bracts minute, about 1 mm. long, .5 mm. broad, subulate, sharply acute, coriaceous. Flowers white, in pairs, bracteate, 5-8 mm. in diameter when fully opened, mature flower buds 2-4 mm. long, pedicellate; pedicels 1-2 mm. long, glabrous; sepals 5, imbricate, 1-1.5 mm. broad, ovate, orbicular, coriaceous with a thin colourless minutely irregularly, toothed or somewhat fimbriate margin; petals 5, white imbricate, slightly coriaceous, subequal, 3 mm. to 4 mm. long, 2-3 mm. broad, oblong, obovate rounded at the tips with thin colourless irregularly minutely toothed or fringed margin, concave, somewhat hooded at the apex, more or less covering the whorl of stamens. *Stamens* 5 inserted on the disk; disk annular, more or less cup-shaped with minutely fibrillated rim; filaments equal, 1-1.5 mm. long, shorter than the petals, slightly flattened at the base; anthers equal, ovate, about 1 mm. long, .5-8 mm. broad. *Ovary* pyramidal more or less, 3-cornered, 1 mm. long, .5 mm. broad at the base; style almost absent; stigma pointed. *Fruit* a capsule, one seeded, 12 mm. long,

6 mm. broad, oblong-ellipsoid, slightly attenuated towards the base and the apex with persistent calyx at the base, and crowned with a hard somewhat conical 1 mm. long persistent stigmatic point.

SOUTH BURMA: Tavoy, fruiting October, Wallich's No. 4339, *Microtropis? longifolia* Wall., 2nd. October, 1827, Nathaniel Wallich—(Type in Calcutta Herbarium). Senyatkokadin chg. Tavoy, flowering March, flowers white, Maung Ba Pe, 7th. March 1932, No. 13105; *Microtropis pachyphylla* Merrill and Freeman, Thaton, Yathey-Taung, alt. 830 m., 24th March 1928, flowers white, Mg. Po Chin No. 6553. Brandis No. nil; Herb. Sulp. Kurz; Amherst District, Kyondo to summit of Dawna Hills via new road from Kawkareik to Thingan-nhin-naung, alt. 900 m. I. H. Burkil, No. 30313, 3rd. March 1908, A. T. Gage, Tavoy, Nos. 9-17, 43; P.T. Russell, Tavoy, No. 41 (now in Kew Herbarium).

This species differs from the allied species *M. filiformis* King, in its thick leathery, acute leaves with indistinct lateral nerves and glaucous undersurface, marginate petiole, divaricate dichotomously branched cymose inflorescence and small (of the size of an almond-nut) woody capsule with persistent calyx and projecting stigmatic point at the apex.

E. D. Merrill and F. L. Freeman in their paper on the "Old world species of the Celastraceae genus *Microtropis* Wallich," published in the *Proceedings of the American Academy of Arts and Sciences*, Vol. 73, No. 10, pp. 298-299, May 1940, considered Mg. Po Chin No. 6553 and Burkil 30313 as new to science and described the species, as noted above, under the name—*M. pachyphylla* sp. nov. They also, from the insufficient data available to them, were of opinion that *M. longifolia* Wall. was distinct from *M. pachyphylla*. The arguments put forward in support of their decision are as follows:

" 42. *Microtropis pachyphylla* sp. nov.

BURMA: Yatheytaung, Theton District, 6553, Mg. Po Chin, Forest Ranger, type, 24th. March 1928 (C, photo. A), a small tree in rocky places, alt. 730 m., flowers white; Amherst District, Dawna Range, Burkil 30313 (C) a small bush with white flowers, alt. about 900 m., 3rd. March 1908, without locality Brandis, ex-herb. S. Kurz (C, photo A.).

This from all specimens originally identified as representing *Microtropis longifolia* Wall., is characterised by its coriaceous, obscurely nerved, pale, verruculose leaves, and differs from Wallich 4339, the type of *Microtropis longifolia* Wall. ex. Kurz in its very much longer, many flowered, much longer peduncled cymes, those of *M. longifolia* Wall. ex. Kurz (in fruit) not exceeding the petioles in length. Although in his original description of Wallich's species, *Jour. As. Soc. Bengal* 42, (2): 65. 18, 1873, Kurz cites only "Wall. 4339 (pro parte)" he mentions the short cymes with peduncles 4 to 6 lines long, and describes the fruits, we feel confident that the form above described with

rather lax cymes much exceeding the petioles in length and with peduncles 1.5 to 3.5 cm. long should not be placed in the same category as Wallich 4339, a fruiting specimen, its short peduncled fruiting cymes scarcely equalling or only slightly exceeding the petioles.

"43. *Microtropis longifolia* Wall., List No. 4339, 1830, *nomen nudum*, Kurz, *Jour. As. Soc. Bengal* 42 (2): 65. 1873 descr., *For. Fl. Brit. Burma* 1: 250. 1877.

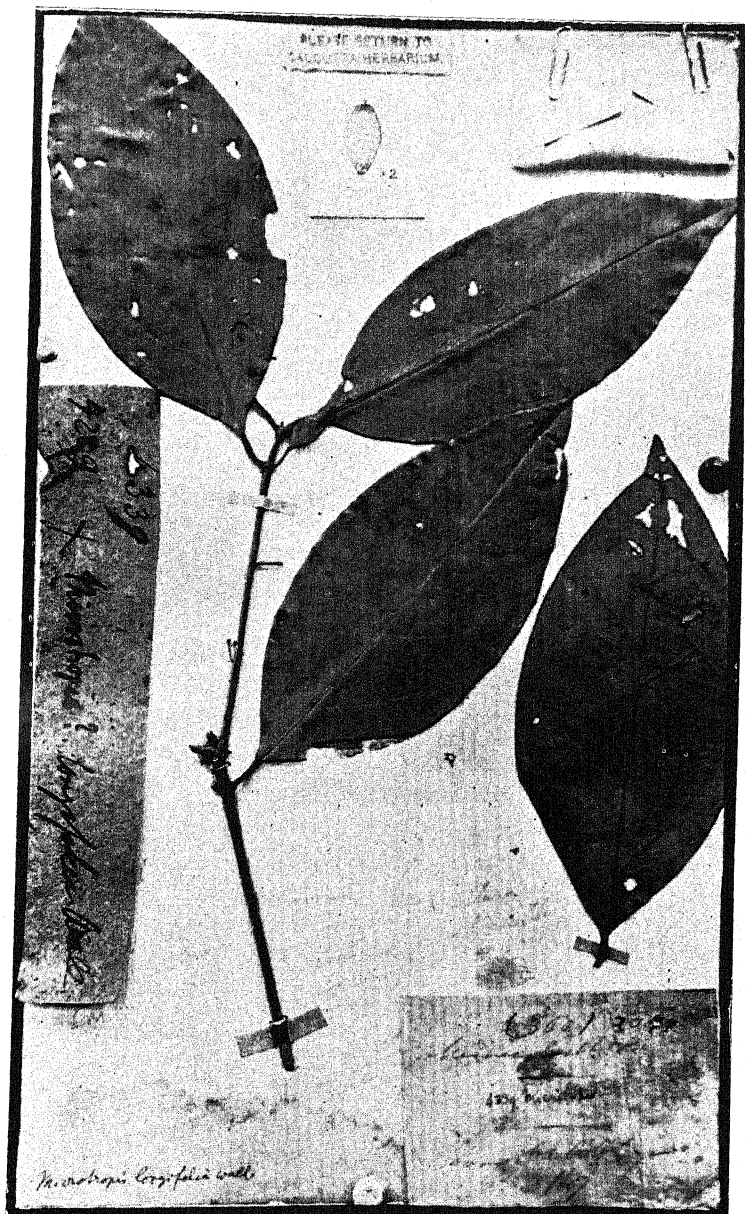
BURMA: Tavoy, Wallich 4339 (Col. Gomez) (C. holotype, photo. and drawing A).

We are constrained to limit this species, for the present, to the type collection, Wallich 4339, with its holotype in the Calcutta Herbarium. Kurz's Latin description of 1873, based on Wall. "4339 (*pro parte*)" and his English one of 1877, appertain to the form here considered and the Wallich specimen in the Calcutta Herbarium was the source of his specific name. Other material named at Calcutta as representing *Microtropis longifolia* Wall. we place under *M. pachyphylla* Merr. and Freem., *supra*. Wallich's species is characterised by its elliptic to oblong-elliptic coriaceous, verrucose, fairly ample, few nerved leaves and its short infructescences which scarcely equal or but slightly exceed the petioles in length. There has been some confusion regarding the species because the Kew sheet, of which we have an excellent photograph, has mounted with it a specimen of *Salacca macrophylla* Wall. (Supposedly = *S. flavescens* Kurz), which however has its own label "(303) 1584, tree. Tavoy, 2nd. October 1827". This accounts for the Index Kewensis entry "*longifolia*, Wall Cat. n. 4339 = *latifolia*, *Salacia flavescens*". The mixture was not in the collection but was due to an error in mounting two independent collections on the same sheet.

We have seen no specimen of Wallich, 4339, our interpretation being based on Kurz's short description and a drawing of the sheet of Wallich's specimen in the Calcutta Herbarium. Mr. Airy-Shaw, who examined the material at Kew, states that he has no doubt as to its representing *Salacia flavescens* Kurz. It may be that the actual specimen in the Calcutta Herbarium represents the *Salacia*; it is a leafy branch with a single detached fruit. In such case *Microtropis longifolia* Wall. would be eliminated from the genus".

It will be obvious that the confusion is due evidently to Merrill and Freeman not having the opportunity of seeing the type sheet of Wallich's *M. longifolia*. Their conclusions are evidently not based on my observations which were communicated to them but reached them too late on account of the international situation. Due to this unavoidable delay they were not able to modify their previous deductions. Therefore the only course now left to me is to reduce, as I have done under my full description of the species, Merrill and Freeman's new species *M. pachyphylla* to *M. longifolia* Wall.





*Microtropis longifolia* Wallich. Photograph of type sheet Wall. Cat. No. 4339, Tavoy, 2nd October 1827. Reduced 2/5th natural size. Drawing of capsule on top is now almost the same size in the photograph.

K. BISWAS—SYSTEMATIC POSITION OF A LITTLE KNOWN  
FLOWERING PLANT FROM SOUTH BURMA





## EFFECT OF ANTHOCYANIN PIGMENTS ON THE RATE OF PHOTOSYNTHESIS IN *ERANTHEMUM* SPP.

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(Communicated by P. Parija)

Received for publication on August 24, 1940

### INTRODUCTION

A LARGE amount of work has been done to study the effect of anthocyanin pigments on chlorophyll though leading to contradictory results. Very little attention has been paid to study its effect on photosynthesis.

Anthocyanin pigments have been regarded to act as a light screen, protecting the underlying chlorophyll from excessive insolation, by workers like Pringsheim<sup>8</sup>, Hussack<sup>6</sup>, Wiesner<sup>16</sup>, and Ewart<sup>4</sup>, Shibata.<sup>12</sup> Shibata and co-workers<sup>13</sup> and Rosenheim<sup>10</sup> also added to this view saying, that the derivatives of flavones when present in peripheral tissues, absorb ultra-violet rays and are thus protective. This light screen theory of anthocyanin met a strong opposition even from earlier workers like Reinke<sup>9</sup> and Englemann.<sup>3</sup> Englemann observed that the red pigment absorbs those rays which are complementary to chlorophyll and hence least harmful to it.

One serious objection to the light screen theory comes from cases when the pigment is in the lowermost layer of cells of a leaf. The screening of light by anthocyanins, if at all done, is possible only when it is present in the uppermost layers. But there are plants, like the present one (*Eranthemum*) where it is mostly found in the lower epidermis of the leaves.

However, in spite of objections, the light screen theory seems to have gained more popularity among the plant physiologists. Probably this popularity of the theory led people to believe that anthocyanins hinder photosynthesis by screening off light from chlorophyll.

But Wheldale<sup>17</sup> observed that anthocyanins appear to accompany lessened photosynthetic activity, as in plants towards the end of their vegetative season, in autumnal reddening of leaves, in an unhealthy condition in evergreens during winter. She also observed that anthocyanin is not readily produced where carbon assimilation is most active.

These observations appear to suggest a possibility, though very remote, of anthocyanin pigments having some property to

make up the deficiency in photosynthesis and hence having some favourable effect on it. But to investigate into the problem directly is very difficult, due to the impossibility of altering at will, any of the internal factors (here anthocyanin content) without effecting any other change in it. As such, the present work is an attempt to correlate the rate of photosynthesis with chlorophyll content in the green and anthocyanin containing leaves separately and then to compare the results thus obtained.

#### MATERIAL AND METHOD

The present investigation was carried on with detached green and purple (containing anthocyanin) leaves of *Eranthemum* Sp. (Garden varieties *Eranthemum albomarginatum* and *E. atropurpureum*). Two such plants, green and purple varieties were planted side by side in the botanical garden attached to this laboratory. Both the plants were almost of the same age and they were growing under identical external conditions.

That the age of a leaf has some effect on the rate of photosynthesis, was observed by Singh and Lal<sup>14</sup> and as such, leaves almost of the same age were taken from both the plants.

Usually leaves fourth, fifth and sixth in position from the buds were used for the present work, because each branch of these plants had leaves only upto the sixth or seventh nodes from the bud.

The leaves were severed under water from the plants by a clean cut at the base of the petiole, with a sharp scalpel. It was done on the previous day usually at about 4 or 5 P.M. They were then kept overnight under a bell-jar with their petioles under water, so that they might take as much water as they could. The leaves were severed in pairs from each node, and one from each pair was used, next day to determine the water content.

Next morning a green and a red leaf from among them were introduced into two separate glass leaf-chambers and kept in a vertical position with their petioles under water. The lids of the leaf chambers were then replaced and sealed airtight with plasticine.

A mixture of air and carbon dioxide, having 5 per cent. of the latter by volume, was passed simultaneously through the two leaf-chambers and a blank tube serving as control. At first, proportionate amounts of the gases were collected in a big aspirator bottle, serving as a gas reservoir. This gas mixture was then forced out of the reservoir by liquid paraffin flowing in from another aspirator bottle placed at a much higher level. These gas streams after passing through the leaf-chamber and the blank tube, were led through Pettenkoffer tubes containing equal quantities of standard baryta water. Finally, the escaping gases were collected in graduated cylinders by displacement of water and their volumes measured. The gas was passed through all the three channels at a constant rate of 500 c.c. per hour.

The work was carried on in a dark room and the light used was a  $\frac{1}{2}$  Watt. 1000 c.p. Phillips electric lamp, placed at a distance of 30 cm. from the leaf-chambers. The heat rays from the lamp were cut off by interposing two water screens, four inches in thickness, through which tap water at laboratory temperature was constantly circulating. These water screens were considerably helped by using a table fan which was blowing a continuous blast of air on the lamp. The exhaust fan of the dark room was also kept rotating at a high speed in order to force out the hot air and have a current of outside air through the room. Care was taken to see that the temperature within the leaf-chambers remained fairly constant throughout the course of one experiment and if any rise of the temperature was noticed, it was immediately brought down by judiciously pouring ice cold water into the water screens.

To start with a fresh pair of leaves, a preliminary run of two hours was given in every case, to pass the period what has been termed as the 'induction period' by Manning.<sup>7</sup> That this induction period in the material used was found to extend nearly upto two hours and after this period the rate of photosynthesis was found to assume more or less an uniform rate (Fig. 1).

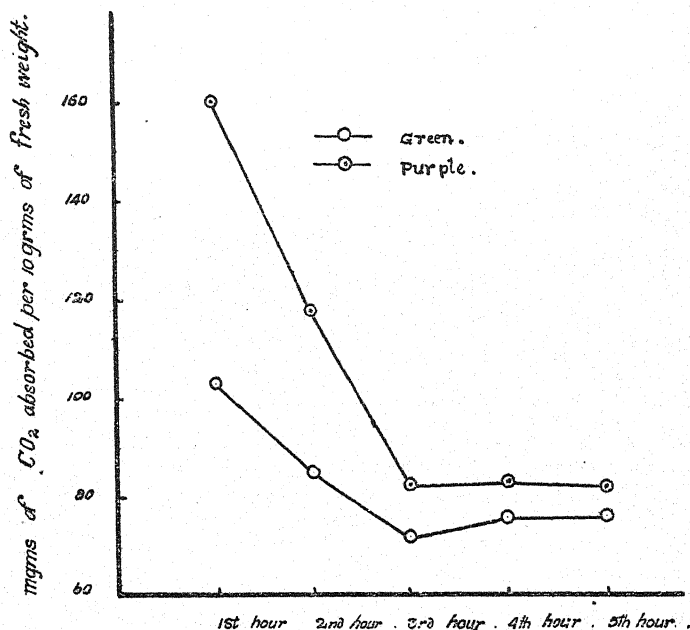


Fig. 1. Course of hourly photosynthesis in purple and green leaves.

However, after a preliminary run of two hours, the experiment was allowed to run for one hour, for one reading, after which the baryta in the three sets was titrated separately against standard hydrochloric acid. The amounts of carbon dioxide absorbed by

the two leaves were calculated from the differences between the titration values of those from the control tube and the tubes attached to the leaf-chambers. To these apparent assimilation values, the values of respiration were added in order to get the real assimilation. The respiration values of the leaves were obtained by taking the mean of the respiration records taken before and after photosynthesis.

After this the leaves were taken out from the chambers and their chlorophyll extracted quantitatively and purified by Shertz's<sup>11</sup> method. The amount of chlorophyll in the saponified solutions were determined colorimetrically by matching them against Guthrie's<sup>5</sup> synthetic standard using Klett's top reader colorimeter.

The internal temperature of the leaves was determined by using copper-tungsten thermocouples and a mirror and scale galvanometer.

#### OBSERVATIONS AND DISCUSSION

It was noted that the rate of photosynthesis in the anthocyanin containing leaves starts with a much higher value than that in the green. It falls also rapidly within the induction period and then takes a more or less steady course (Fig. 1). But when a study of hourly readings of photosynthesis, as alternating with hourly respiration was made, it was observed that in both the leaves the fall in the rate of photosynthesis is much slower than in the previous experiment and in the green leaf the course becomes steady from the very beginning (Fig. 2).

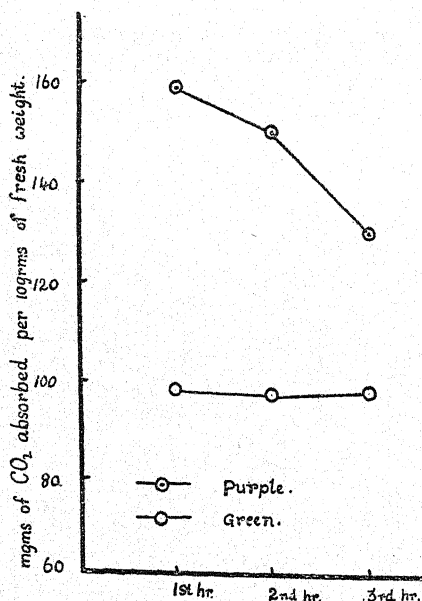


Fig. 2. Shows the rate of hourly photosynthesis as it alternates with hourly respiration in purple and green leaves.

The experiment was spread over a period of two months during which there was a considerable fluctuation of temperature, ranging from 26° C to 33° C. The photosynthetic rate also fluctuated considerably as it may be seen from Table III that at the lowest value of the temperature the assimilation number was minimum and similarly at the highest value of the temperature it was maximum in both the cases.

The results of this experiment when subjected to usual statistical calculations of correlation and regression show that the correlation coefficient 'r' of the photosynthetic rate and chlorophyll content is higher in the anthocyanin containing leaves by 0.02 than that of the green leaves (Tables I and II). From Fig. 3, it may also be seen

TABLE I

Observation Nos.	Chlorophyll in mg.	Observed photosynthesis in mg.	Estimated photo. in mg.	Error
1	2.26	13.4	15.498	+2.098
2	2.88	15.6	20.136	+4.536
3	2.52	16.6	17.443	+0.843
4	2.54	17.0	17.592	+0.592
5	3.08	20.8	21.632	+0.768
6	2.04	14.4	13.852	-0.548
7	1.94	12.8	13.104	+0.304
8	1.53	10.6	10.038	-0.562
9	3.10	21.8	21.731	-0.019
10	1.76	11.6	11.758	+0.158
11	2.70	19.8	18.789	-1.011
12	2.92	21.0	20.435	-0.565
13	2.74	21.2	19.094	-2.126
14	1.88	14.5	12.656	-1.844
15	2.24	18.2	15.348	-2.852

S. E. XY of estimate = 3.2

'b' = 7.48

Y = 1.468 - 7.48 X

(regression equation for the computation of the estimated values)

'r' = 0.77

Observed 't' = 8.225

Calculated 't' from Fishers 't' table at 13 degrees of freedom at .01 level of significance = 3.012.

TABLE II

Observation Nos.	Chlorophyll in mg.	Observed photosynthesis in mg.	Estimated photo. in mg.	Error
1	2.14	8.2	11.969	+3.769
2	2.34	8.6	12.995	+4.395
3	2.10	11.0	11.764	+0.764
4	3.72	11.2	20.940	+8.874
5	2.68	14.0	14.739	+0.739
6	3.48	18.4	18.853	+0.453
7	3.02	16.8	16.484	-0.136
8	3.42	19.2	18.535	-0.665
9	1.92	11.2	10.840	-0.358
10	2.02	11.4	11.350	-0.046
11	2.70	15.6	14.842	-0.758
12	2.54	13.6	14.021	+0.421
13	3.70	20.2	19.971	-0.229
14	3.12	17.8	16.640	-1.160
15	2.32	14.0	12.892	-1.107
16	3.76	22.2	20.280	-1.920
17	2.92	17.6	15.970	-1.630
18	3.20	20.2	17.407	-2.793

S.E. XY of estimate = 2.85

'b' = 5.13

Y = 0.991 + 5.13 X

(regression equation for the computation of the estimated values.)

'r' = 0.75

Observed 't' = 12

Calculated 't' from Fisher's 't' table at 16 degrees of freedom at .01 level of significance = 2.921.

that the regression of photosynthetic rate over chlorophyll content is linear and the difference between the observed and the calculated values do not exceed the standard error of estimates, except in two cases of green leaves.

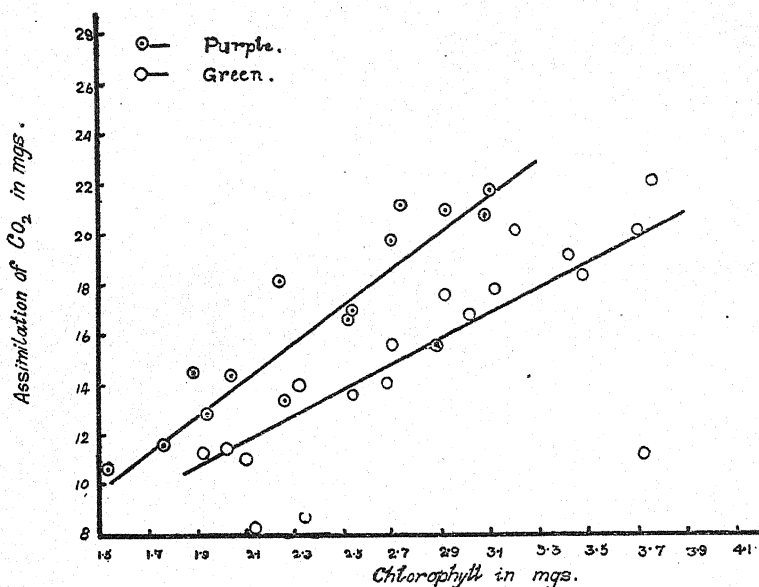


Fig. 3. Regression of photosynthetic rate on chlorophyll content in purple and green leaves. The upper line shows the course in purple leaves and the lower one shows that in the green leaves.

The above findings show that the rate of photosynthesis per unit of chlorophyll is higher in the anthocyanin containing leaves than the green ones.

Willstatter and Stoll<sup>18</sup> in their classical works on chlorophyll as internal factor controlling photosynthesis found that the yellow leaves of *Ulmus* showed same rate of photosynthesis as the green ones though their chlorophyll content was much lower than that of the green. In the present case the chlorophyll content of the green leaves on the average, was 1.2 to 1.3 times higher than that of the purple ones. The assimilation number in the purple leaves varied between 6 and 8, while that in the green variety between 3 and 6. There was a difference of 1.63 between the mean values of these two samples taken. Since there was so much of variation in between the individual observations made, it was considered worthwhile to see whether this difference between the means was really significant or not. In order to test its significance Fisher's 't' test for

small samples, i.e., 't' =  $\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$  was made. It may be seen

from Table III, that the observed 't' was more than twice higher than the calculated 't' in Fisher's Table at 0.01 per cent. level of significance. As such the difference between the two means does not seem to be casual but a real one.



TABLE III  
Assimilation Numbers

Observation Nos.	Purple	Green	Temperature in°C.
1		3.83	26-27
2		3.67	26-26.5
3	5.92	5.23	27-28
4		3.01	26-26.5
5	5.41	5.22	27-28
6	6.59	5.29	29.5-30.5
7	6.69	5.56	30-30.5
8	6.75	5.61	30-31
9	7.06	5.83	30-31
10	6.60	5.14	30-30.5
11	6.93	5.78	31-31.5
12	7.03	5.35	30-30.5
13	6.57	5.46	30-30.5
14	7.33	5.71	31-32
15	7.19	6.03	31-32
16	7.73	5.90	32.5-33
17	7.71	6.02	32-33
18	8.12	6.31	32-33

S.E. of the two means = 0.8246  
 Observed 't' = 5.66  
 Calculated 't' from Fisher's table = 2.5  
 P = .01

Moisture content of a leaf has been claimed to be one of the internal factors controlling photosynthesis. Dastur<sup>2</sup> found that leaves with higher moisture content photosynthesise more rapidly than those with lower moisture content. In the material taken for the present investigation, the moisture content after the overnight treatment was found to be fluctuating between 83 to 86 per cent. Besides the average moisture content of the green leaves was only 1.3 per cent. higher than that of the anthocyanin containing leaves. In the present material which contains more than 80 per

cent. of water, this slight difference may not account for any difference in the photosynthetic rate in the two leaves and even if it did interfere instead of inhibiting, it would have raised the rate in the green ones above that of the purple leaves.

Anthocyanin has also been regarded as a means to raise the temperature by workers like Comes and Kerner,<sup>1</sup> Smith<sup>15</sup> and Wulff.<sup>19</sup> According to Wulff the pigment is very important for arctic plants for it absorbs extra radiant energy and thereby raises the temperature of the organs in which it is found to occur. In the present instance also the anthocyanin containing leaves showed a slightly higher internal temperature, than the green ones higher only by 0.4° C. to 0.6° C. But it is quite evident that this small difference in temperature alone cannot account for such a difference in the photosynthetic rate in the two kinds of leaves, for according to Vant Hoff,  $Q_{10}$  will be near about 2.5.

The rate of respiration in the anthocyanin-containing leaves has always been found to be much higher than that in the green ones. So it was thought desirable to see whether this high rate of respiration in the purple leaves, was helping the rate of photosynthesis by adding to the supply of carbon dioxide which might be acting as a limiting factor. For this purpose three different concentrations of carbon dioxide 5, 6 and 7 per cent. were used with the same leaves. But there was no significant difference in the rate of photosynthesis neither in the green nor in the purple under the three different concentrations of carbon dioxide. Thus the carbon dioxide content in the gas was not limiting the rate of photosynthesis.

Taking all the above facts into consideration it is evident that the anthocyanin pigments in the leaves have a favourable influence on the photosynthetic rate.

#### SUMMARY

The correlation of photosynthetic rate and chlorophyll content was studied in the green and anthocyanin containing leaves of *Eranthemum* spp.

Photosynthesis was determined by continuous gas current method and chlorophyll was estimated colorimetrically.

The anthocyanin containing leaves showed a higher photosynthetic rate than the green ones, in spite of lower chlorophyll content. The results were statistically significant.

The anthocyanin containing leaves showed a slightly higher internal temperature than that in the green ones.

At the start—the anthocyanin containing leaves show a much higher rate of photosynthesis than the green ones and falls rapidly taking a steady course after 3 hours (Fig. 1).

The author expresses his grateful thanks to Professor P. Parija, M.A. (Cantab.), I.E.S., for his kind and helpful guidance throughout the course of this investigation. The author is also indebted to the

Government of Orissa for the research scholarship granted to him and also to Dr. G. B. Banerjee and the Physics Department for lending him the thermocouples and a galvanometer.

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## ON *HORMIDIELLA*, A NEW MEMBER OF THE *ULOTRICHACEÆ*\*

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Received for publication on August 12, 1940

THIS alga came up in a culture of some soil algae at Madras in July 1938. At the time of its appearance there was plenty of *Phormidium* sp. and *Chlorococcum humicolum* (Naeg.) Rabenh. growing in the culture. Soon after its appearance, the alga became very dominant and the *Phormidium* and the *Chlorococcum* gradually became less and less prominent, and, after a month or so, completely disappeared. The alga was sub-cultured into other bottles. It grew well in these cultures and generally occupied the moist sides of the bottles immediately above the level of the culture solution, but a fair quantity of the alga was found at the bottom and also on the surface of the culture solution. The culture solution used was Moore and Karrer's (1919, p. 285) solution which was slightly modified by the addition of 0.005 per cent. of urea. The culture solution had the following composition:—

Ammonium nitrate	..	..	0.5 gm.
Potassium acid phosphate	..	..	0.2 gm.
Magnesium sulphate	..	..	0.2 gm.
Calcium chloride	..	..	0.1 gm.
Iron sulphate	..	..	trace
Urea	..	..	0.05 gm.
Distilled water	..	..	1000 c.c.

The detailed development of the alga was followed in hang-drop cultures made from the main cultures.

### DESCRIPTION

The alga is filamentous and resembles a *Ulothrix* or a *Hormidium*, but its lowermost cell always possesses a long hyaline thread-like stalk with a knob-like disc at the end by means of which it is attached to the substratum. Its cells are short and cylindrical, often cask-shaped, and are 8–9  $\mu$  broad, and 3.2–8  $\mu$  long. The thread-like stalk of the basal cell is 3.5–5.26  $\mu$  long. Each cell possesses a single nucleus and a plate like parietal chloroplast with a pyrenoid embedded in it (Text-fig. 2). The chloroplast extends the full length of the cell (Text-fig. 4) and, in vertical view, almost completely encircles the cell. The lateral cell wall consists of two

\* From the University Botany Laboratory, Madras.

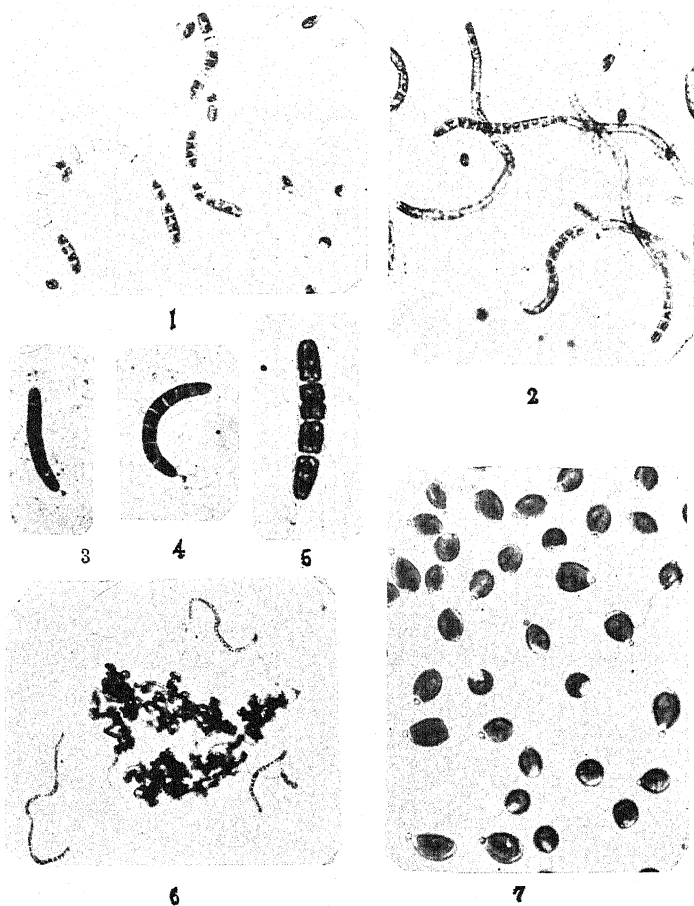
layers, a thin firm outer layer and a slightly thicker inner layer. The transverse wall is thin, consisting of only a single layer and is continuous with the inner layer of the lateral wall (Text-fig. 3).

Both the cell wall and the thread-like stalk give clear cellulose reaction when tested with iodine and sulphuric acid. They are not stained with ruthenium red showing that there is not much pectic material in them. The apical cell of the filament is broadly rounded at the top and the lowermost cell of the filament is slightly narrowed towards the base. At first, the filaments in the cultures were quite short, and generally did not contain more than about 16 cells, and were either straight or slightly curved (Pl. V, Fig. 2, Text-figs. 1 and 4). But in older cultures, the filaments were very much longer and were moreover very much twisted (Pl. V, Fig. 6). The length of the filament in the younger cultures ranged between 135-168  $\mu$  and in the older cultures between 206-250  $\mu$ .

#### ASEXUAL REPRODUCTION

Asexual reproduction takes place by means of zoospores which are formed singly in each cell (Text-figs. 5-7, 10-12, 18). The zoospores are slightly dorsiventral, being somewhat convex on one side and slightly concave or flat on the other (Text-figs. 5 b, 10). Each zoospore possesses two cilia which are attached at the anterior end somewhat laterally towards the concave side (Text-fig. 8). The two cilia are equal in length and are placed very close to each other. A small, somewhat thick greenish refractive body could be often seen near the base of the cilia (Text-figs. 8, 9). Whether this represents the blepharoplast or not, it could not be decided, as the body was single and not double and was moreover situated a little to one side of the place of attachment of the cilia. A single, somewhat obliquely oval, plate-like chloroplast in which is imbedded a pyrenoid occupies the posterior portion of the zoospore (Text-figs. 5 a, b, 8-10). The anterior portion is hyaline and has a somewhat dull refractive appearance. Owing to this refractive appearance, no contractile vacuoles could be seen even when examined very carefully under higher magnifications. A few small granules are present in this portion. No eye-spot could be seen, even though numerous zoospores were carefully examined. In a few zoospores however, a tiny reddish speck was seen very close to the base of the cilia. Whether this represented a rudimentary eye-spot could not be decided. The fact that the swarm spores do not travel very far from the mother-plant, but settles down close to it very soon after liberation may be in some way connected with the poor development of the eye-spot. The zoospores are 5-5.5  $\mu$  broad and 6.65-7  $\mu$  long. The cilia are about 10.2  $\mu$  in length.

The zoospores, in their dorsiventral shape, in the somewhat lateral position of the two cilia and in the absence of an eye-spot, show a very close resemblance to those of *Hormidium* as figured by Klebs (Klebs, 1896, Taf. II, Figs. 23 and 24). Two contractile vacuoles were observed by Klebs in *Hormidium* in the anterior hyaline portion, but as already pointed out, owing to the refractive

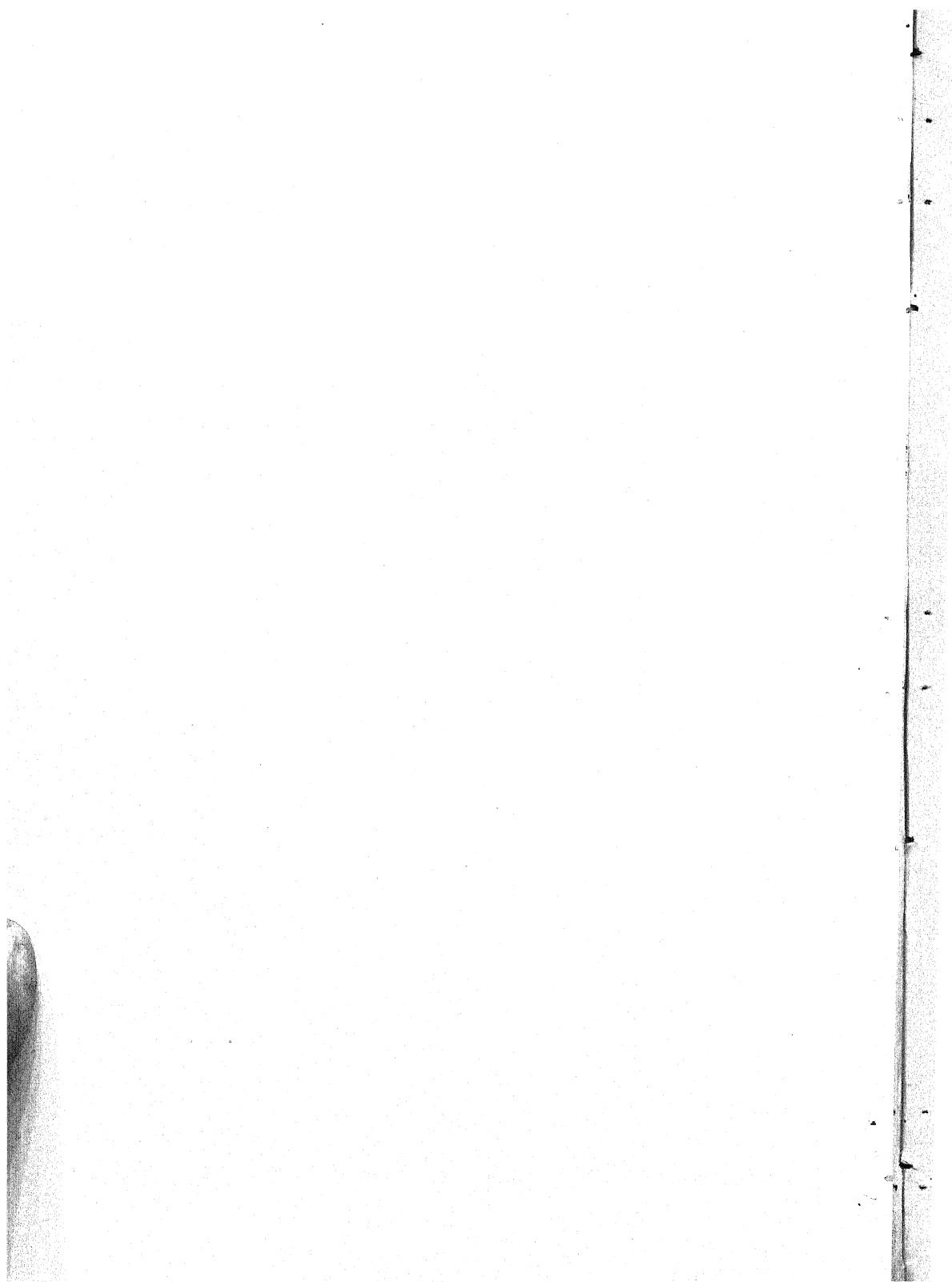


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*HORMIDIELLA PARVULA* GEN. ET. SP. NOV.



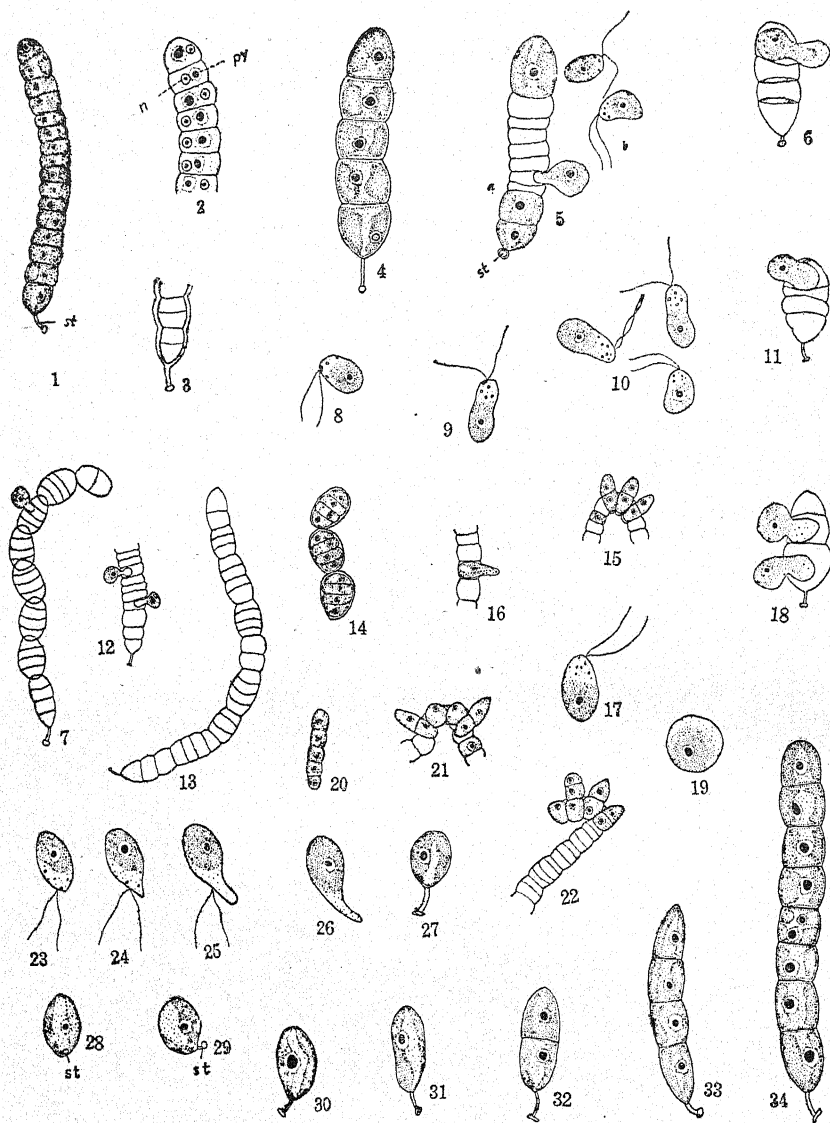
nature of the anterior portion, no contractile vacuoles could be made out in the present alga. This refractive substance is evidently connected with the formation of the attaching stalk as may be seen later on in the paper (see pp. 161-162).

The zoospores generally come out between 8-30 and 9 A.M. A single zoospore is formed in each cell. It escapes through a round aperture formed in the lateral wall of the cell. Since this aperture is very narrow, the zoospore has to squeeze itself out through it very gradually (Text-figs. 5, 6, 7, 11, 12, 18). At one stage, it looks as though the zoospore would be cut into two (Text-figs. 6, 12, 18). But this never happens and the zoospore escapes from the cell quite alright every time. The method of escape of the zoospore through a very narrow opening closely resembles that of *Hormidium flaccidum* as figured by Klebs (Klebs, 1896, Taf. II, fig. 21). Usually zoospores escape out with their posterior end foremost (Text-figs. 11, 18), but frequently they escape with their anterior end foremost also (Text-fig. 6). The time taken for the zoospore to escape from the cell is about 10 to 15 minutes. Very commonly practically whole filaments get nearly or completely empty (Pl. V, Fig. 1, Text-figs. 7, 12, 13). It is very interesting to see the whole of the hang-drop culture full of active motile zoospores. It may be pointed out here that in this alga, unlike in *Ulothrix*, the basal cell also is able to form zoospores (Text-fig. 18). Plenty of zoospores were formed in the young cultures. But in the older cultures zoospore-formation was seen only very occasionally and that only from a few cells of the filaments.

Prior to the formation of the zoospores, the cells of the filaments divide into 2 or 4, or occasionally, 3 smaller cells by one or two successive transverse divisions (Text-figs. 7, 13, 14). And from each one of these smaller cells a single zoospore is formed. It may be pointed out here again that the basal cell, unlike in *Ulothrix*, is able to divide like the rest of the cells of the filament (Text-figs. 7, 18). This division of the mother-cell usually takes place about 3 to 4 A.M. An interesting phenomenon is observable before this cell division takes place. A few hours previous to the cell-division, say about midnight or so (11-30 P.M. to 1 A.M.), the protoplast of the mother-cell begins to show peculiar movements inside the cell. Sometimes the protoplast would get massed together on one side and sometimes on the other and this movement will continue for quite a long time, until finally the cell division takes place.

As soon as the zoospores are liberated, they have a somewhat elongated shape with a slight depression on the concave side (Text-figs. 5 b, 10), but they soon become more ellipsoid (Text-figs. 5 a, 8, 10). They swim at first with an irregular wobbling forward movement, often also in circles, but a few minutes later, the forward movement is slowed down and the movement becomes more or less rotatory and wobbling and confined to a very narrow area. After about 10 to 15 minutes, the movement becomes very slow and the zoospores finally become completely quiescent or show only slow





Text-figs. 1-34. *Hormidiella parvula* gen. et. sp. nov. Fig. 1. Full grown filament, thread-like stalk and disc-like attaching disc at the end ( $\times 275$ ). Fig. 2. A portion of a filament showing the nucleus and the pyrenoid in the cells ( $\times 820$ ). Fig. 3. A portion of a filament stained with gentian violet showing the two layered nature of the cell-wall ( $\times 450$ ). Fig. 4. A short filament; note the plate-like parietal chloroplast extending the full length of each cell ( $\times 780$ ). Figs. 5-7, 11-13, and 18. Showing the escape of zoospores from the cells of the filament; note the escape of the zoospore from the basal cell in fig. 18. (Figs. 7, 12 and 13  $\times 275$ ; the rest  $\times 780$ ). Figs. 5a, b, 8, 9 and 10. Zoospores; note the refractive body

oscillatory movements. Their cilia at this stage continue, however, to be vibratile.

#### GERMINATION OF THE ZOOSPORES

The zoospore, after becoming quiescent, soon forms from its anterior end an elongated thread-like stalk by means of which it attaches itself to the substratum (the cover glass of the hang-drop culture) (Text-figs. 23-27). The way in which the attaching stalk is formed could not be made out for quite a long time. At first we thought that the two cilia of the zoospore became fused together along their length and formed a thread-like attaching stalk, but later on we found that this presumption was wrong. After a few days of careful watching, we were able to observe the actual details of the formation of this stalk. When the zoospore becomes quiescent, its anterior end is close to the lower surface of the cover-glass in the hang-drop culture. The cilia, however, are seen still actively vibratile. After a short time a protuberance is formed rapidly from the anterior portion of the zoospore a little to one side of the region of the attachment of the cilia (Text-fig. 24), and the protuberance soon grows longer and longer, thus forming the narrow elongated thread-like stalk (Text-figs. 25, 26). As the stalk is being formed, the protuberance at first appears to be somewhat short and beaked and hollow with a small quantity of protoplasm inside (Text-figs. 25, 26). But very rapidly, the protuberance becomes longer and at the same time loses its hollow appearance and becomes narrower and solid, its protoplasmic contents evidently receding into the main portion of the zoospore. The end of the stalk becomes somewhat expanded into a disc-like structure by means of which it attaches itself to the cover-glass (Text-fig. 27). The cilia could be seen clearly for a short time after the formation of this protuberance (Text-figs. 24, 25), but, very soon after that, the cilia could not be seen at all. We were unable to find out whether the cilia are discarded outside or are absorbed inside the body of the zoospore. We searched carefully near the zoospore for the possible presence of the discarded cilia, but were unable to find any. It seems therefore very probable that the cilia are retracted inside the body of the zoospore. As the attaching stalk is formed, the hyaline portion of the zoosporic cell decreases in size and at the same time loses its refractive appearance and becomes quite transparent. This is evidently due to the fact that the substance which made the

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near the base of the cilia (blepharoplast?) in figs. 8 and 9 ( $\times 780$ ). Fig. 14. Division of the cell into four daughter-cells before the formation of the zoospores ( $\times 275$ ). Figs. 15, 16, 21 and 22. The formation of aplanospores and their germination ( $\times 235$ ). Fig. 20. A short filament formed from a single aplanospore; note the absence of the stalk in the filament ( $\times 235$ ). Fig. 17. A gamete (?) ( $\times 780$ ). Fig. 19. A gamete (?) which has rounded itself, after losing its cilia ( $\times 780$ ). Figs. 23-27. Showing the stages of formation of the stalk by the zoospore as it settles down ( $\times 780$ ). (Figs. 24-26. Diagrammatic.) Figs. 28-34. Stages showing the development of the zoosporic germling into full grown filament ( $\times 780$ ).  $n$  = nucleus;  $py$  = pyrenoid;  $st$  = stalk.

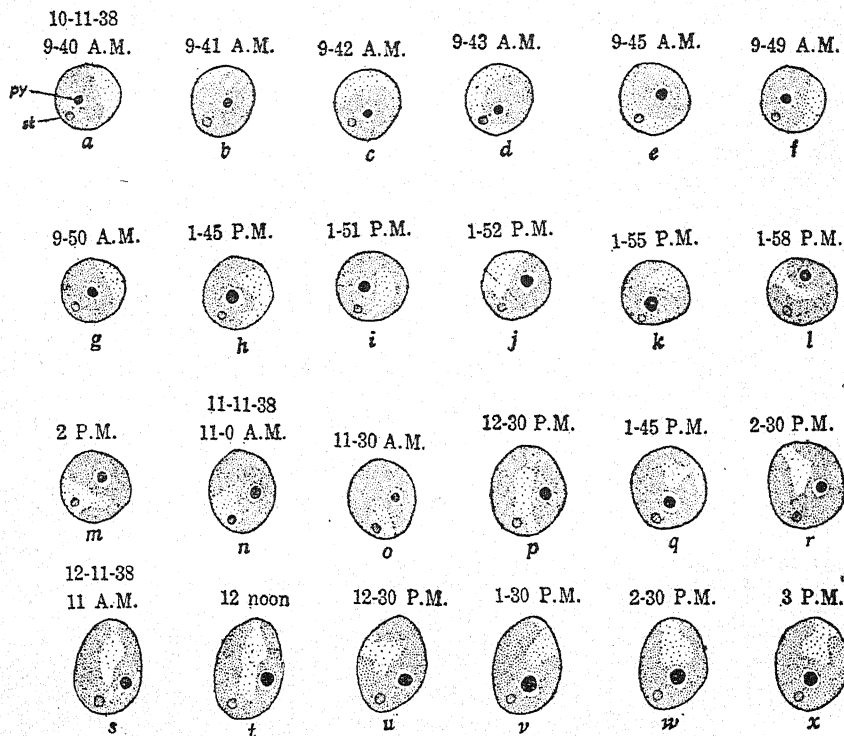
anterior portion of the zoospore very refractive was very probably used up in the formation of the attaching stalk with the result that the refractive anterior portion becomes quite transparent.

In the hang-drop cultures the stalk of the zoospore is not visible at first and the zoosporic germling appears as if attached to the cover-glass directly by a small disc-like knob (Pl. V, Fig. 7; Text-figs. 28, 29). This is because the long stalk is hanging straight from the cover-glass downwards and is therefore not visible when viewed from above. The stalk becomes visible if the cover-glass with the hang-drop is mounted on a glass slide and gently pressed on, when the stalk, owing to the pressure, lies flat on the slide and is then very well seen (Text-figs. 27, 30 and 31).

The thread-like stalk of the alga resembles very much that of *Ophiocytium majus* Naeg. It is very interesting to note that a certain amount of resemblance is seen even in the manner of formation of the stalk in these two algæ. Pascher (1925, p. 73) states regarding the formation of the stalk in *Ophiocytium majus* "Zunächst leigen (immer nach Scherffel) die Schwärmer noch unbeweglich vor der Zelle und schwimmen dann ruhig ohne Rotation davon. Bei der Keimung kugelt sich der vordere Teil ab, während aus dem hinteren, von dichter weiszglanzender Substanz erfüllten Teile ein stielformiger Fortsatz herausmodelliert wird, der später zum Stielchen wird. Die Geisselpartie und das Vorderende sind nach Scherffel bei der Keimung nicht beteiligt." But the stalk in *Ophiocytium majus* is formed by the posterior end of the swarm-spore, whereas, in the present alga, it is formed by the anterior end. This similarity in the formation of the stalk in these two algæ does not of course mean anything more than a mere case of parallel development, since *Ophiocytium* belongs to the Xanthophyceæ, while the present alga belongs to the Chlorophyceæ.

Soon after the formation of the attaching stalk, a wall is formed round the protoplast of the zoospore. This protoplast of the germling is, however, not quiescent, but shows a very peculiar movement inside the wall continuously for quite a long time (up to 48 hours or more). As a result of this movement, the position of the chloroplast could be seen continuously shifted inside the cell. After about 48 hours the movement becomes slower and finally stops. The movements of the protoplast of a single zoospore germling was followed by means of camera lucida drawings for three days and the continuous changes of position of the contents are shown in Text-fig. 35 a-x. On the first day, immediately after the zoospore settles down (at about 9 A. M.), the position of the chloroplast was changing almost every five minutes (Text-fig. 35 a-g). But towards the evening the change of position of the chloroplast became slower and thereafter still slower. Again the shape of the chloroplast also shows a gradual change. On the morning of the first day the chloroplast was more or less plate-shaped (Text-fig. 35 a-g), but towards the evening, it became somewhat bell-shaped (Text-fig. 35 h-m). On the third day or so, the cell became somewhat elongated and the

chloroplast became a curved plate extending nearly the whole length of the cell, and in vertical view encircling the cell almost completely (Text-fig. 35 s-x).



Text-fig. 35 (a-x) *Hormidiella parvula*.—Showing the protoplasmic movement inside a single zoosporic germling. a-m protoplasmic movements observed on 10-11-1938 from 9-40 A.M. to 2 P.M.; n-r movements observed on 11-11-1938 from 11 A.M. to 2-30 P.M.; s-x movements observed on 2-11-1938 from 11 A.M. to 3 P.M.  $\times 1330$ . py = pyrenoid; st = stalk.

The zoosporic germlings after reaching a length of about  $18-20\mu$ , divided into 2 cells by a transverse wall and by further divisions grew into filaments of about 8 cells (Pl. V, Figs. 3-5; Text-figs. 31-34). These filaments then produced zoospores in their turn. The time taken for the zoospores to grow into full filaments and form zoospores in their turn was about 17-20 days.

#### APLANOSPORES

Some of the zoospores do not escape from the cells, but surround themselves with a thin membrane while still inside the mother wall and become aplanospores. These aplanospores soon grow out into short filaments of 2 or 3 cells (Text-figs. 15, 16, 21, 22). These short filaments grow at right angles to the mother filament and give a false appearance of lateral branching (Text-figs. 15, 16, 21, 22).

But an examination of different stages of these growths clearly shows their real nature. When these short filaments are about 2 or 3 cells long, they become detached from the mother plant and grow into new independent filaments. The filaments which are formed by the aplanospores do not possess an attaching stalk (Text-fig. 20).

Mention must be made here of some peculiar swarm-spores which were formed in some of the older cultures. These swarm-spores resembled in all respects the ordinary zoospores but were slightly larger than the latter (Text-fig. 17). They, unlike the normal zoospores, do not settle down soon after their escape from the mother plants (*i.e.*, within 10–15 minutes), but keep on swimming for quite a long time (two hours or longer) and finally become quiescent. They then lose their cilia and become rounded. They do not form an attaching stalk (Text-fig. 19), nor do they get attached to the substratum (cover-glass) in any other manner. They soon show signs of disintegration and finally die. The significance of these peculiar larger swarm-spores is not clear. The fact that they do not settle down and grow into new plants suggests that they are probably not zoospores. It is just possible that they represent gametes (macrogametes ?) which do not find partners.

#### VEGETATIVE REPRODUCTION

The filaments, both in the earlier and in the older cultures, often become fragmented into smaller bits of one or more cells. These then by the division of their cells grow into longer filaments. The filaments which grow out of the fragmented bits do not show any attaching stalk.

#### DISCUSSION

This alga at first sight looks like a *Ulothrix* with a peculiar thread-like attaching stalk, developed from its basal cell. The chloroplast also is like that of *Ulothrix* in being a curved parietal plate and extending the whole length of the cell and also in encircling the cell almost completely. But its swarm-spores are dorsiventral and quite unlike those of *Ulothrix*, but more like those of *Hormidium* (Klebs, 1896, Taf. II, Figs. 24, 28). And its basal cell, unlike in *Ulothrix*, can divide and produce swarm-spores like the other cells of the filament. The present alga differs from *Hormidium* in the following respects. The chloroplast in the latter genus is generally a parietal plate with a circular or elliptical outline and does not extend the full length of the cell, but occupies more or less the centre of it, and moreover, does not encircle more than half of the cell (Printz, 1927, p. 166; Fritsch, 1935, p. 205; Smith, 1933, p. 384; Klebs, 1896, p. 328; Heering, 1914, p. 41). In the present alga, on the other hand, the chloroplast is a curved plate, and extends the full length of the cell and also encircles almost the whole of the cell. Again, in *Hormidium* no rhizoidal cell is formed, and there is no difference between a base and an apex (Klebs, *loc. cit.*, p. 343; Printz, *loc. cit.*, p. 166; Heering, *loc. cit.*, p. 41), whereas in the

present alga, a definite stalk is formed by its basal cell, and the differentiation of a base and an apex is a very characteristic feature of the alga.

It may thus be seen that the present alga combines the features of both *Ulothrix* and *Hormidium* and, in addition, possesses a feature peculiar to itself in having a thread-like stalk which is formed in a very characteristic manner. It forms an interesting link between *Ulothrix* and *Hormidium* and, therefore, may be placed in a new genus which may be called *Hormidiella*.

*Hormidiella* gen. nov.

Thallus filamentous and unbranched and consisting of a row of cells, and attached to the substratum by the basal cell; apical cell broadly rounded at the top; basal cell with a long thread-like stalk below, by means of which the filament is attached to the substratum; the remaining cells, cylindrical or cask-shaped and more or less uniform in appearance; all cells including the basal cell capable of division and of producing swarm-spores. Cells uninucleate; chromatophore parietal, plate-shaped, with a single pyrenoid in it; chromatophore extending the full length of the cell and in vertical view almost completely encircling the cell. Reproduction by means of biciliate zoospores formed singly in each cell; zoospores dorsiventral, with two equal cilia attached somewhat laterally towards the ventral side; eye-spot absent. Aplanospores occasionally formed. Sexual reproduction not known.

*Hormidiella parvula* sp. nov.

General characters same as those of the genus, cells 8-9  $\mu$  broad and 3.2-8  $\mu$  long; stalk of the basal cell 3.5-5.26  $\mu$  long. Filaments either straight or slightly curved; in older cultures intricately twisted or contorted; zoospores 5-5.5  $\mu$  broad and 6.65-7  $\mu$  long.

*Hab.*—In a laboratory culture of soil algæ at Madras.

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## EXPLANATION OF PLATE V

- FIG. 1. Filaments with plenty of empty cells after the escape of the zoospore; note the single celled zoosporic germlings.  $\times 140$ .
- FIG. 2. Full grown filaments.  $\times 140$ .
- FIGS. 3-5. Young filaments showing the attaching stalk.  
Figs. 3 & 4  $\times 280$ ; Fig. 5  $\times 540$ .
- FIG. 6. Filaments from older cultures showing a contorted growth.  
 $\times 40$ .
- FIG. 7. Zoosporic germlings two hours old attached to the cover glass by means of their stalk.  $\times 510$ .

## A NOTE ON *ULOTHRICHOPSIS VIRIDIS* GEN. ET SP. NOV. \*

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AND

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Received for publication on August 12, 1940

THIS alga was found in a culture of soil algae growing along with *Chlorococcum humicolum*. The culture was made by inoculating 5 grams of paddy-field soil from Tambaram near Madras in Moore's solution. Since the alga looked very interesting an attempt was made to study it in further detail by growing it in hang-drop cultures.

The alga consists of very short unbranched filaments made up of one to three or occasionally four cells placed in a row. Its cells are  $15.8-33.3\ \mu$  long and  $6.2-7.9\ \mu$  broad. At first sight it looks like a *Stichococcus* but an examination of the cell-contents shows that it is not a *Stichococcus*. Each cell has one or more plate-like chloroplasts, in which are embedded one or more pyrenoids. The usual number of chloroplasts is one, two or four (Figs. 1-5, 13), but occasionally 8 chloroplasts are seen in some of the cells. The number of chloroplasts is largest just before cell division. A single nucleus is situated in the centre of the cell (Figs. 10-12). The cell-wall is thin and uniform.

The normal method of multiplication of the alga is by fragmentation. The filaments, after reaching the 4 or 3-celled stage, were observed to break up very rapidly into shorter lengths of one or more cells (Figs. 8, 13-17).

No zoospores or gametes were observed in the living material, though it was kept under observation in culture for over 3 months. Very occasionally the contents divided into a few round masses inside the cell wall. Quite a number of cells showing these round masses were found in the material (Figs. 16-17). These masses did not escape outside, though they were watched for a long time. They are evidently aplanospores of the alga.

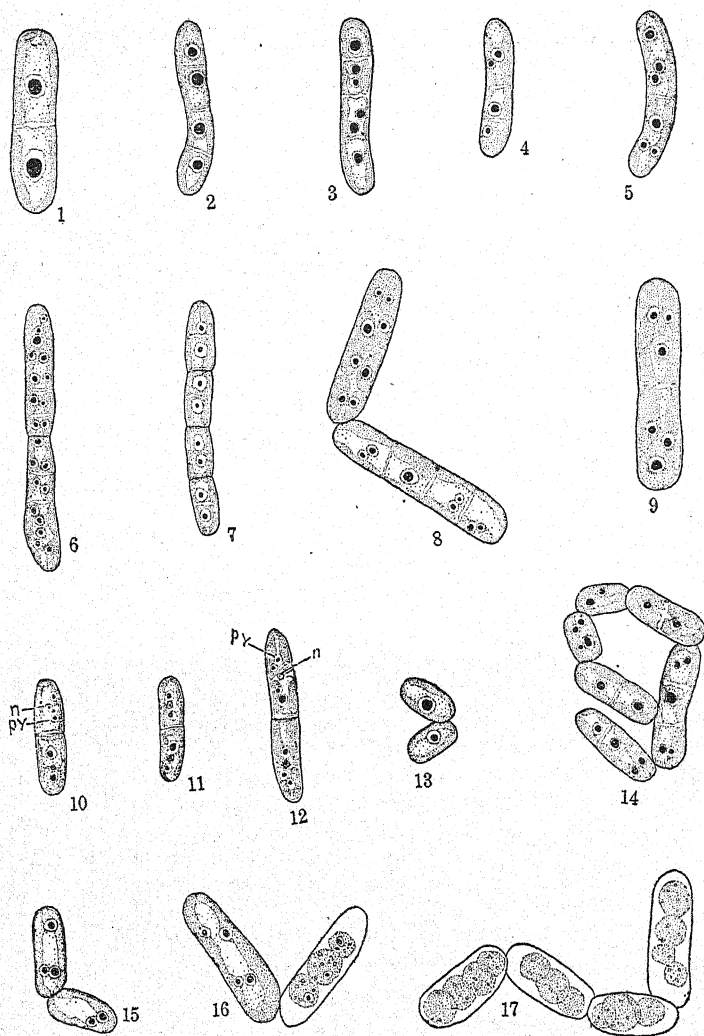
### SYSTEMATIC POSITION OF THE ALGA

The alga with its bright green plate-like chloroplasts containing one or more pyrenoids in each looks at first sight very much like an Ulotrichaceous alga. On closer examination, it is found that each cell usually contains several (1-8) chloroplasts (Fig. 8). But no member of the Ulotrichaceae is known to possess more than a single

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\* From the University Botany Laboratory, Madras.





Text-figs. 1-17. *Ulothrichopsis viridis* gen. et. sp. nov. Fig. 1. A single cell with two chloroplasts, each with a pyrenoid. Figs. 2-4. Cells with 3 or 4 chloroplasts; Fig. 3. shows 2 pyrenoids in the two central chloroplasts. Fig. 5. Cell showing 3 pyrenoids in each chloroplast. Fig. 6. A short 2-celled filament. Fig. 7. A four-celled filament. Fig. 8. A 2-celled filament; the upper cell shows 8 plate-like chloroplasts. Fig. 9. A single cell with chloroplasts just divided. Figs. 10-12. Stained preparation showing the nucleus and pyrenoid. Figs. 13-15. Filaments breaking apart into individual cells. Figs. 16 and 17. Formation of aplanospores. (Figs. 1, 8, 9, 16 & 17  $\times 800$ ; rest  $\times 430$ ).

chloroplast in each cell.† The alga shows a close resemblance to *Heterothrix exilis* Pascher (*Bumilleria exilis* Klebs), (Klebs, 1896, p. 389, Taf. II, Figs. 15-30; Bristol, 1920, pp. 78-79, Text-fig. 1; Pascher 1932, p. 344, Figs. 22b-28c). Only, in the present alga, pyrenoids with a starch sheath are present, whereas in *Heterothrix exilis* no pyrenoids are present as in all the Xanthophyceæ. But Korshikov (1930) has recently shown that pyrenoids are present in the chloroplasts of another member of this genus, viz., *Bumilleria sicula* Borzi. And Klebs (1896, pp. 224, Taf. I, Figs. 17-19) has shown that pyrenoids are present in the chloroplasts of young plants of *Botrydium granulatum*. But the pyrenoids of both these algæ do not show any starch sheath round them. Printz (1927, p. 409), however, says that in Xanthophyceæ in general, pyrenoids and starch are absent. Pascher (1925, p. 116) also, when referring to the pyrenoids in *Botrydium*, writes "Ebenso bedarf die Pyrenoid-frage dringendst". In the case of the present alga, a very definite starch sheath is present around the pyrenoids. Testing with iodine shows the starch layer clearly stained dark blue. And preparations stained in iron-hæmatoxylin show a white unstained ring representing the starch sheath around the darkly stained pyrenocrystal. In the face of the current view regarding the absence of starch in the Xanthophyceæ, the presence of a definite starch sheath around the pyrenoids in the present alga forms an insuperable difficulty in referring it to *Heterothrix*, an accepted member of the Xanthophyceæ. But, since pyrenoids, though without a starchy layer, have been shown to be present in young plants of *Botrydium* by Klebs (1896) and in *Bumilleria sicula* by Korshikov (1930), the possibility of the occurrence of starch also round the pyrenoids in some of the members of the Xanthophyceæ should not be ignored. But unfortunately the main point which ought to decide the position of the present alga, viz., the nature of the cilia of the swarm-spores, is still unknown. If this should be known, then it would be an easy matter to decide whether the alga belongs to the Xanthophyceæ or not. So, until the swarm-spores of the alga are known, it would be best to consider the alga as only an Ulotrichaceous one and to place it in a new genus by name *Ulothrichopsis* close to *Ulothrix*. If, at a later date, the swarm-spores of the alga should be found and prove to be heterokonton, then the alga will have to be transferred to the Xanthophyceæ as a new species of *Heterothrix*.

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† The genus *Sphaeroplea*, which has been recently included by Fritsch (1935, p. 222-26) in the Ulotrichales, would appear to form an exception to the rule, since it contains a large number of ring-shaped chloroplasts in each cell. But this is a special case. The larger number of chloroplasts has been explained as probably due to a failure of cross-wall formation between the several cells, each containing a single chloroplast. *Sphaeroplea* according to this view should be considered as having been derived from a septate Ulotrichaceous type which has ceased to form a septa except at rare intervals.

## DESCRIPTION

*Ulothrichopsis* gen. nov.

Thallus filamentous and unbranched, consisting of a few cells only placed in a row; each cell containing a single nucleus and one or more parietal plate-like chloroplasts with one or more pyrenoids in each. Vegetative reproduction by fragmentation of the filaments into shorter lengths consisting of one or more cells. Asexual reproduction by aplanospores. Zoospores or gametes unknown.

*Ulothrichopsis viridis* sp. nov.

General characters same as those of the genus. Filaments one to four cells placed in a row; cells  $6.2-7.9\ \mu$  broad,  $15.8-33.3\ \mu$  long. Chloroplasts 1-8 (usually 2-4) in each cell.

*Hab.*—In a laboratory culture of soil algæ from Tambaram, near Madras.

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ON THE LIFE-HISTORY OF *CHARACIUM*  
*TERRESTRIS* SP. NOV.\*

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(Communicated by M. O. P. Iyengar)

Received for publication on August 12, 1940

THE alga was found growing along with *Phormidium* sp. and *Oscillatoria* sp. in some cultures of soil algæ at Madras. It is unicellular ovoid or ellipsoidal and grows attached to the substratum by a more or less refractive thread-like stalk, the lower end of which is expanded into a small disc at the point of attachment. The cell contents are dense and green and it is not easy to make out the nature of the chloroplast (Figs. 1, 21-23). A single pyrenoid is imbedded in the chloroplast. Six to seven nuclei are seen around the pyrenoid in stained preparations (Figs. 2-3). Even young plants are coenocytic and contain 2-3 nuclei. The mature cell without the stalk is  $26.2-38.5 \mu$  long and  $22.8-38.5 \mu$  broad; the stalk is  $7-10 \mu$  long.

The cell-wall consists of two layers, an inner thin and delicate layer immediately next to the protoplast and an outer thicker and firm layer. The two layered nature is not very clear in the ordinary cell, but is seen very clearly in empty cells after the zoospores have escaped. Both these two layers show a cellulose reaction with iodine and sulphuric acid. These two layers are also stained deeply with ruthenium red showing that they contain plenty of pectic material as well. The stalk on the other hand shows only the cellulose reaction with iodine and sulphuric acid and does not get stained with ruthenium red showing that it is made up mainly of cellulose, without much of pectic material.

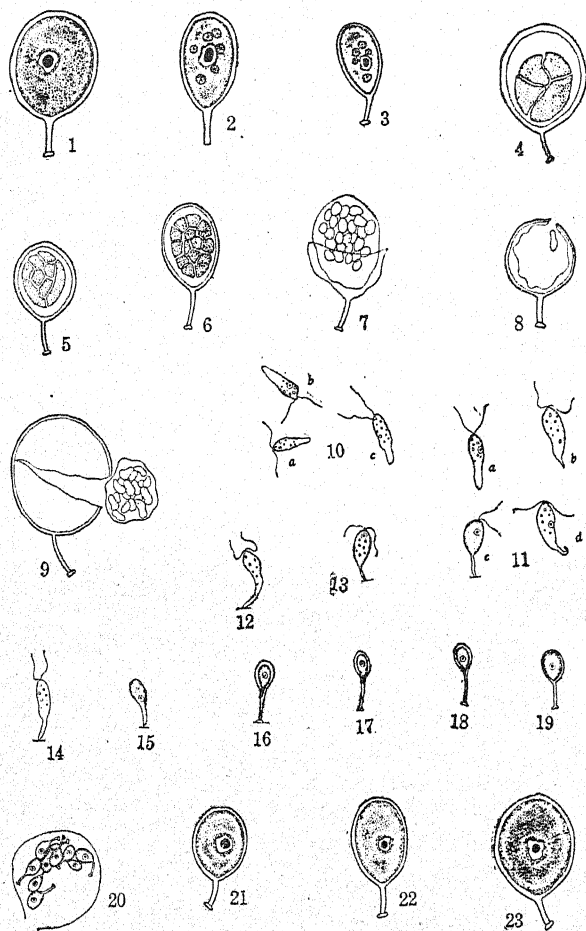
Asexual reproduction takes place by means of zoospores. Each cell by the division of its contents gives rise to about 32-64 zoospores. The first indication of the formation of the zoospores is several cleavages of the cytoplasm. The zoospores are generally liberated in the morning between 8-30 and 10 A.M. Occasionally they are liberated during mid-day also. They are biciliate and more or less conical in shape with a broadly rounded anterior end and a gradually narrowed posterior end (Figs. 10 and 11). The posterior portion is slightly hyaline and is often produced into a small tail. A very tiny papilla is present at the anterior end. The contents of the zoospore are highly granular. The chloroplast is not definite but diffuse. No pyrenoid is present. A round eye-spot is seen

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\* From the University Botany Laboratory, Madras.

This paper formed part of a Thesis approved for the Degree of Master of Science of the University of Madras.

at the lateral region. The contractile vacuoles could not be made out clearly. A small round vacuole containing a granule is present in the anterior portion but the vacuole is not contractile (Fig. 10 b). The zoospores are  $11.2-16.0\ \mu$  long and  $3.2-4.8\ \mu$  broad; the cilia are about  $8\ \mu$  long.



Text-figs. 1-23. *Characium terrestris* sp. nov. Fig. 1. Fully grown cell. Figs. 2 and 3. Cells stained in hæmatoxylin showing the nuclei. Figs. 4-6. Cells undergoing cleavage before the formation of zoospores. Figs. 7 and 9. Zoospores escaping from the cell enclosed in a vesicle. Fig. 8. An empty cell after the escape of the zoospores. Fig. 10. a, b, c. Zoospores. Figs. 11-14. Zoospore just coming to rest; cilia still present. Figs. 15-19. Stages in the germination of the zoospores. Fig. 20. Autosporous inside the vesicle. Figs. 21-23. Fully grown sporelings. All Figs.  $\times 440$ .

The zoospore escapes out of the mother-cell through the rupture of the cell wall across the middle or at the top (Figs. 8, 9). The cell

never gets ruptured at the stalk end. The zoospore before escaping changes its shape and position inside the mother-cell very often; it is sometimes round, sometimes elongate and sometimes contorted and finally assumes its normal shape when it escapes out. It escapes generally with the broad ciliary end foremost, but occasionally it escapes by the tail end also. Zoospores either come out one after the other or in groups of two or three. Sometimes the whole mass of zoospores covered with a thin delicate vesicle is extruded out of the mother-cell, and the zoospores then escape one by one from the vesicle.

As soon as the zoospores are liberated, they swim away from the mother-cell and are active for a short time, but during that period they swim very fast and it is very difficult to follow them. They swim with the ciliary end foremost, but the thin narrow tail end also shows a peculiar wagging movement. After swimming for about 20 minutes or so, they settle down and attach themselves to the substratum. The way in which the zoospore settles down and attaches itself to the substratum is extremely interesting and was followed in hang-drop cultures. After swimming for a time, the zoospore becomes quiescent at one place, but the cilia can be clearly seen actively vibratile. The hyaline posterior portion gets gradually still further elongated and finally becomes a thin thread-like stalk. The extreme end of the stalk attaches itself to the substratum and becomes somewhat disc shaped (Figs. 11 and 14). The cilia can be seen slowly moving at the anterior end until the stalk is completed, and then finally disappear. Whether they are discarded or absorbed inside, it could not be made out.

A young germling about a few minutes old is  $6-9\ \mu$  long without the stalk and  $5.2-8\ \mu$  broad. The stalk is about  $8\ \mu$  long. The germling becomes a full grown plant in about a fortnight and then produces a crop of zoospores in its turn.

#### AUTOSPORES

Occasionally the zoospores fail to escape from the mother-cell, and develop inside the mother-wall like the ordinary germlings with a normal stalk (Fig. 20). Sometimes the zoospores which are extruded out in a mass fail to separate themselves, and develop inside the vesicle for sometime. They become free only after growing for some time and reaching a certain size.

#### DISCUSSION

This alga, in being unicellular and attached to the substratum by its stalk-like lower end belongs to the genus *Characium*. Its mode of asexual reproduction also shows that it is a *Characium*. It shows, however, many interesting and peculiar features which have not been so far observed in any of the previously recorded species. The mode of attachment of the zoospore to the substratum does not appear to have been observed clearly before. Smith (1916, pp. 464-65) states with regard to the zoospores of *Characium Sieboldii*, A. Br.

"Since stages in the liberation of the young zoospores were not observed the manner of their coming to rest could not be determined. From our knowledge of other algæ it would seem probable that they always come to rest with the cilia downward". The mode of attachment of the zoospores of the present alga by their narrow hyaline posterior end is extremely peculiar and quite unlike most other algæ. Coming to the mode of formation of the stalk of the alga, this appears to be very similar to that of *Ophiocytium majus* Naeg. as observed by Schiller (Pascher, 1925, p. 73). According to him the anterior part of the zoospores of *Ophiocytium majus*, during germination, becomes rounded while from the posterior portion is formed a rod-like process filled with a refractive substance. This rod-like process becomes later on the stalk of the alga. The similarity in the formation of the stalk in these two such unrelated genera, one belonging to the Chlorophyceæ and the other to the Xanthophyceæ, is a very interesting instance of parallel development.

In the mode of attachment of the zoospore to the substratum by its posterior end, the present alga resembles *Chlorangiopsis anomala* Korshikov (1932, pp. 581-82, Taf. X, Figs. 40-44), but these two algæ differ from each other in all other respects, since *Chlorangiopsis* is more nearly allied to *Chlamydomonas* than to *Characium*.

The alga appears to be a new species and may be called *Characium terrestris* sp. nov.

*Characium terrestris* sp. nov.

Cells shortly stalked, obovate to nearly globose,  $22.8-38.5\ \mu$  broad,  $26.2-38.5\ \mu$  long without stalk; stalk narrow and filamentous  $7-10.5\ \mu$  long and expanded at the point of attachment into a tiny disc. Zoospores biciliate and conical with a broadly rounded anterior end and a narrow elongate posterior end,  $3.2-4.8\ \mu$  at the broadest part and  $11.2-16\ \mu$  long.

*Hab.*—In a laboratory culture of soil algæ at Madras.

The author wishes to express her great indebtedness to Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., for his constant guidance and help during the course of this investigation and in the preparation of this paper. Her sincere thanks are also due to the authorities of the University of Madras for the award of a research scholarship during the tenure of which the present investigation was carried out.

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ON THE REPRODUCTION OF *ANADYOMENE*  
*STELLATA* (WULF.) AG.\*

(PRELIMINARY NOTE)

BY M. O. P. IYENGAR, M.A., PH.D. (LOND.), F.L.S.

AND

K. R. RAMANATHAN, B.Sc. (HONS.), M.Sc.

Received for publication on October 15, 1940

THIS alga was collected at Krusadai Island near Pamban and was brought in a living condition to Madras and kept growing in the laboratory in Schreiber's culture solution made up in sea-water. The plants produced plenty of swimmers on a number of days. Two types of plants were found, one set of plants producing quadriciliate zoospores and the other forming biciliate gametes. The two types of plants were externally quite similar and could not be distinguished from one another. The quadriciliate zoospores after swarming for a short time, settled down and began to germinate. The biciliated gametes fused in pairs and formed plenty of zygotes. The fusion was generally isogamous but frequently slightly anisogamous also. The gametes from the same thallus did not fuse, the fusion always taking place between gametes from different thalli.

Very little is known regarding the reproduction of *Anadyomene*. The only detailed account of the reproduction in this genus is by Derbes and Solier (1850) who have described the formation of swimmers in the cells of the alga. But they do not mention whether the swimmers are gametes or zoospores, nor do they mention the number of cilia possessed by the swimmers. No cilia are shown even in the swimmers figured by them (Derbés and Solier, 1850, Pl. 32, fig. 9). Later authors (De Toni, 1889; Collins, 1909; Wille, 1911; Printz, 1927 and Fritsch, 1935) merely mention that swarm-spores are formed in the cells of *Anadyomene*.

The present investigation is interesting in showing that there are two types of swimmers, *viz.*, quadriciliate zoospores and biciliate gametes, and that these two types of swimmers are formed on different plants which are externally indistinguishable from one another. The occurrence of two types of plants, one asexual and producing four-ciliated zoospores and the other sexual and forming biciliated gametes, suggests the possibility of the existence of an alternation of an asexual with a sexual generation as in the case of some members of the Cladophoraceæ and Ulvaceæ. The fact that the

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\* From the University Botany Laboratory, Madras.



gametes from the same plant in the present alga do not fuse suggests that the sexual plants are probably dioecious. This appears to be the first record of sexual reproduction in *Anadyomene*. In fact no sexual fusion appears to have been actually observed so far in the Valoniaceæ (Fritsch, 1935, p. 424).

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## BOOK REVIEW

## INDIAN LABIATÆ

A revision of the Labiatæ of the Indian Empire by Dr. S. K. Mukerjee, M.Sc., Ph.D. (Edin.), in Records of Botanical Survey of India, Vol. XIV, No. 1 (1940); published by Manager of Publications, Delhi, India. Price Rs. 5 or 8 shillings.

A recent volume of the Records of the Botanical Survey of India contains a revision of the Labiatæ of the Indian Empire. The author, Dr. S. K. Mukerjee, M.Sc., Ph.D., Curator of the Herbarium, Royal Botanic Garden, Sibpur, Calcutta, is to be congratulated for this fine and complete work. The need for such revision of the larger plant families of India are constantly being felt and this volume should form an eye-opener to Indian morphologists and taxonomists. What stood in the way of Indian taxonomist so far was certainly not inability, but it was lack of encouragement, absence of training in taxonomy, the disadvantage of handling first class herbarium materials. Fortunately the present author had all the requirements necessary to his advantage. From my intimate association with the present work in India and Great Britain where the author and myself have worked together side by side at Edinburgh, Kew, and British Museum of Natural History for a period extending over two years I may now note that Dr. Mukerjee had first class training in these herbaria and the constant encouragement of our common Professor Sir William Wright Smith.

The speedy publication of the monograph was mainly due to the interest taken by Dr. K. Biswas, Superintendent, Royal Botanic Garden, Calcutta.

Our present knowledge of Indian plants have increased considerably during the period of last fifty years. In the light of modern researches hundreds of plants, new to India and new to science, are being discovered and described. Many new genera have been founded and these gradually need to be incorporated in standard works. It is felt that such revisions of many Indian families like the present one are becoming imperative for our work.

The present work consists of 236 pages, including an useful index of eight pages. First ten pages of the monograph are devoted to general information about the distribution, dominance and endemism of the various groups of Labiatæ found in India and abroad. About 218 pages are devoted to description of species and on keys to genera and species. The information and descriptions available in the "Flora of British India" (Vol. IV) have been greatly supplemented in the present work. Thirteen genera new to India have been incorporated. These are:—*Ceratanthus* Muller ex G. Taylor; *Chamaesphacos* Schrenk; *Eurysolen* Prain; *Lagochilus* Bunge; *Microtoena* Prain; *Mollucella* Benth; *Nosema* Prain;

*Paralamium* Dunn; *Paraphlomis* Prain; *Rubitencriis* Kudo; *Satureia* Linn; *Zataria* Boiss; *Ziziphora* Linn. The number of addition of species will be about sixty which include eleven new species described by the author.\*

As is to be expected the monograph fully meets our anticipation in furnishing more complete information about the Indian Labiatæ. The keys to the genera and species are carefully drawn up and seem to be very practical.

The skill of a taxonomist is shown best in preparing key of larger genera and in this the author has done very well indeed. Personally, I liked the introduction of regional distribution to differentiate closely allied species. In this the author has followed some of the continental taxonomists, specially Pax and Hoffmann and their works in *Pflanzenreich*. Keys to some difficult genera like *Plectranthus* (p. 37), *Pogostemon* (pp. 66-67), *Napeta* (pp. 118-21) and *Leucas* (pp. 163-65) have been drawn with particular care and in this the author should deserve special credit. It is in the keys of these genera that regional factors have helped in making them practical and useful.

The endemic content of such a large family will be of interest and according to the author 50 per cent of the species are endemic in India. My estimate of the Indian Labiatæ was much higher and it was 62 per cent with 260 species endemic in India and Burma.†

The relationship of the Indian Labiatæ has not been clearly indicated in the monograph although exhaustive data as regards number of species and genera in surrounding countries have been given. This has been stated in my paper (*l.c.*) where a map has also been given to bring out these affinities. It seems that species of the dry Deccan can be linked with those of Western Himalayas and both these groups forming a majority should find their closest relations with the species of the Orient, through Afghanistan and Persia. The representative genera of the humid eastern India (Assam and Burma) may be *Gomphostemma*, *Mesona* and *Ceratanthus* and these have little or no relationship with species from Deccan, West Himalayas or the Orient. The affinities of the eastern genera should be found with similar groups from China and Malayasia.

The complete absence of any figure and diagrams in a monographic work of this nature is to be deplored. It would have been very desirable to publish the figures of some of the new species described or of those whose figures are not easily available. One or two maps showing the range of distribution of some dominant northern genera (like *Nepeta* and *Salvia*) with contrasting south

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\*S. K. Mukerjee, "A decade of new species of Labiatæ from India, Burma and Tibet," *Notes Roy. Bot. Gard. Edin.*, 1938, 95, 303 and *Tencrium Annandalei*; Mukerjee Sp. Nov. in the present monograph 219.

†D. Chatterjee, "Endemic Flora of India and Burma," *Jour. Roy. As. Soc. Beng. (Science)*, 1939, 5, 51.

Indian genera (like *Leucas* and *Pogostemon*) would have been interesting. A diagram showing the interrelationship of the larger genera would have been also valuable.

On the whole we all should congratulate Dr. Mukerjee for bringing out this complete work. I consider the author to be the living authority in Asia on this family, and we all should expect that he would continue his studies critically on some of the allied families. Plant taxonomist in India should follow Dr. Mukerjee and take up similar works instead of writing small piecemeal papers simply of observational nature. What is wanted is solid intensive work like the present one which was based on the examination of 20,000 herbarium sheets in the Herbaria of Sibpur, Kew, British Museum and Edinburgh.

Every botanical institute in India and abroad should possess a copy of this valuable monograph.

D. CHATTERJEE.

18-8-1940.



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Names of genera and species should be underlined and will appear in italics. The names of the authors of genera and species should always be given.

Original papers must conclude with a summary, drawing attention to the main facts and conclusions. References to literature cited should, as far as possible, be complete and must be carefully verified. A bibliography should be given at the end of the paper arranged alphabetically under authors' names.

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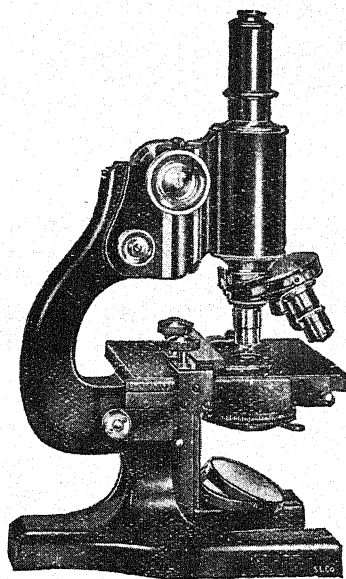
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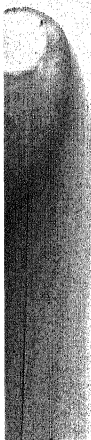
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# The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

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VOL. XIX]

DECEMBER, 1940

[No. 4

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## A CONTRIBUTION TO THE LIFE-HISTORY OF *COSTUS SPECIOSUS* SMITH

BY I. BANERJI

*Department of Botany, Calcutta University*

Received for publication on July 13, 1940

*Costus speciosus* Smith, a plant belonging to the family Zingiberaceæ, is often grown as an ornamental plant in many gardens. It occurs all over the province of Bengal except the Sunderbans, and is easily recognised in its natural habitat by its stout leafy stems often attaining a height of eight feet and bearing dense ovoid spikes with red bracts and white tubular flowers. It is a perennial shrub which flowers in Bengal during the monsoon. According to Prain<sup>12</sup> no other species of *Costus* is found in this province. Hooker<sup>5</sup> mentions the occurrence of *Costus globosus*, *C. Kingii* and *C. speciosus* in India.

The earlier literature on the embryology of the plants of the family Zingiberaceæ has been reviewed by Schnarf<sup>13</sup> and it need not be summarised here. Since then the following important publications have appeared.

Bœhm<sup>2</sup> has described the embryology of *Nicotia atropurpurea* and *Costus cylindricus*. Gregory<sup>4</sup> has studied the development of the female gametophyte in *Elettaria cardamomum*. Mauritson<sup>9</sup> has recently given a comprehensive account of the embryology of a number of plants of the order Scitamineæ and Venkateswarlu<sup>17</sup> of *Phrynium capitatum* of the family Marantaceæ.

The literature on the cytology of the family Zingiberaceæ is very meagre. The chromosome number of a few plants has been determined by Siguara,<sup>15</sup> Morinaga<sup>10</sup> and others. Bœhm<sup>2</sup> has described the development of the microspores in *Nicotia atropurpurea* and recently Gregory<sup>4</sup> has described in some detail the process of microsporogenesis in *Elettaria cardamomum*. No other literature besides the above has been noted by the present writer.

### MATERIAL AND METHODS

The material used in this investigation was obtained from plants grown in the University College experimental garden. Flower

buds in various stages of development were fixed in Allen's modified Bouin's, Licent's, Flemming's (weak) and La Cour's (2 BE) fluids. Of these Flemming's and Bouin's fluids gave very satisfactory results. An exhaust pump was used to ensure thorough penetration of the fixing fluids and the material was fixed in the field during the hottest part of the day. Dehydration and clearing were done in the customary way. The material was imbedded in paraffin and sections were cut 8-12 microns thick depending on the stage required for study. Heidenhain's iron-alum hæmatoxylin and Newton's iodine-gentian-violet were the stains chiefly used.

#### OBSERVATIONS

(i) *The development of the microspores*.—The origin of the sporogenous cells could not be made out definitely in *C. speciosus*, when first noted the sporogenous cells appear to be bigger than the surrounding cells and are situated deep inside the anther tissue. The cells surrounding the sporogenous tissue do not show any evident differentiation into tapetal and parietal cells at this stage.

The microspore mother-cells are mostly polygonal in outline and are closely packed together inside the anther loculus without any intercellular space. In the resting stage the nucleus which is almost spherical, contains a faintly staining reticulum disposed mostly towards the periphery (Pl. VI, Fig. 1). The nucleolus which occupies a more or less central position shows the presence of a small globular bud-like structure which either remains attached to it or gets detached and lies near it. Not more than one nucleolus is present in each cell and it is big in size. At this stage the cells surrounding the sporogenous tissue which have become binucleate push inside the anther cavity. These are the tapetal cells and their protrusion inside the microsporangium appears to be a characteristic feature in Zingiberaceæ as it has been noted by Böhm<sup>2</sup> in *Costus cylindricus* and by Gregory<sup>4</sup> in *Elettaria cardamomum*.

With the onset of heterotypic prophase the microspore mother-cells increase considerably in size and assume a somewhat oblong shape with rounded ends and show signs of rounding off. Inside the nucleus a number of irregularly coiled threads are seen which on close examination show that they are composed of two closely intertwined chromonemata (Pl. VI, Fig. 2). These present a somewhat beaded appearance due to the formation of diamond areas by the intertwined threads. The leptotene threads next conjugate in pairs and the paired threads lie irregularly inside the nuclear cavity (Pl. VI, Fig. 3). At this stage it is not possible to make out the chromonematic structure of the chromosomes. The synizetic knot is not very tight and occupies about half the space inside the nucleus. In most preparations, the nucleus which lies towards the periphery shows the presence of a bud which is almost equal to it in size (Pl. VI, Fig. 4). The contracted knot gradually opens out and lies distributed throughout the nuclear cavity. The diplonema stage next follows. At diakinesis maximum contraction of the

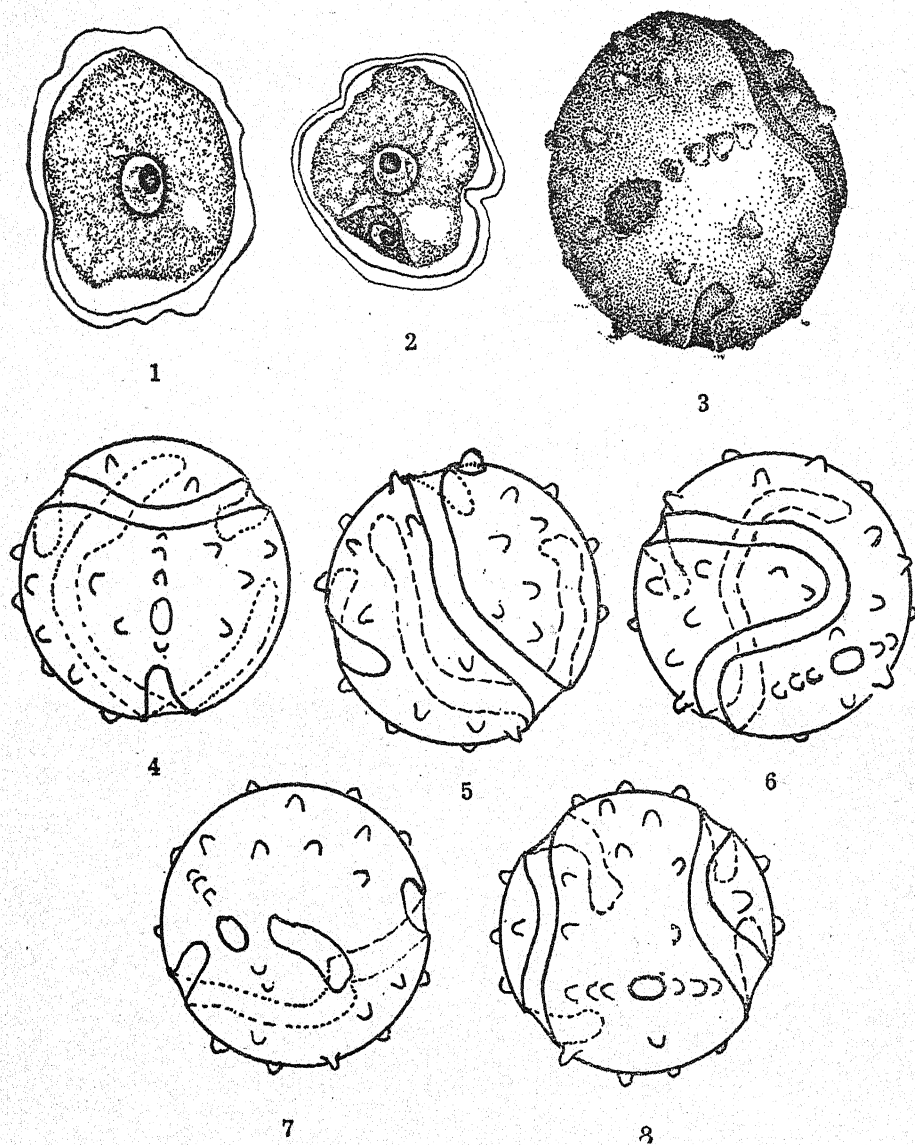
bivalents takes place and the gemini which are somewhat irregular in form lie mostly at the periphery of the nucleus. Two small nucleoli of equal size are noted at this stage which disappear later (Pl. VI, Fig. 5). During division I, a well developed spindle is formed but the fibres do not converge at the poles (Pl. VI, Fig. 6). The bivalent nature of the chromosomes is quite evident. A polar view of an equatorial plate shows 18 chromosomes which, however, show slight size differences (Pl. VI, Fig. 8). The anaphasic separation of the chromosomes appears to be quite regular and no laggards have been noted. As the chromosomes reach the poles they at first mass closely and then open out and become evident, but nothing could be said about their structure on account of their minute size (Pl. VI, Fig. 7). Nuclear membranes are secreted and the nuclei pass into the interkinetic stage. At this stage a phragmoplastic appearance of the protoplast is first noted. A web of fibres occur in the equatorial region of the spindle, but this is very soon replaced by a clear line, simulating a longitudinal split of the cell plate (Pl. VI, Fig. 7).

The II division is normal; the spindles are well defined and they are mostly oriented parallel to each other (Pl. VI, Fig. 9). Polar view of equatorial plates shows clearly the presence of 18 chromosomes. On the completion of division, cell plates appear in the equatorial region of the spindle and quadripartition of the protoplast is thus completed (Pl. VI, Fig. 10).

As noted by Gregory<sup>4</sup> in *Elettaria cardamomum*, the microspore tetrads always show the isobilateral arrangement (Pl. VI, Fig. 10). The liberation of the microspores takes place by the splitting of the cell plates.

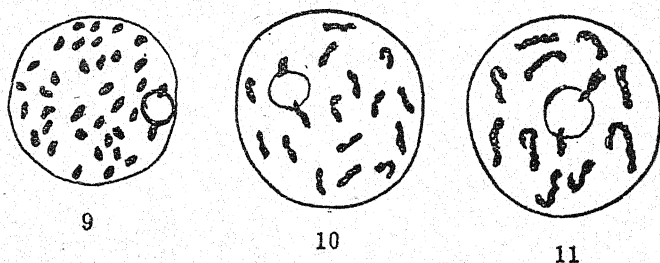
(ii) *The microspores*.—The microspores when liberated from the tetrad are more or less triangular in shape but very soon they increase in size and assume a spherical form and the exine also becomes differentiated (Text-fig. 1). The nucleus at first occupies a central position in the cell but before division it passes on to the periphery. A lens-shaped generative cell is cut off. The nucleus of this cell is smaller than that of the vegetative cell (Text-fig. 2). Further division of the generative nucleus has not been observed and so it could be inferred that the microspores are shed at the two-nucleate stage.

The mature pollen grains are more or less spherical when fully swollen, and their average diameter is 111.4 microns. A single elliptical germ pore is present the margin of which is somewhat fimbriate (Text-fig. 3). Furrows are generally two in number and mostly unequal in size. Sometimes the longer one is branched in the form of Y or V. The various types of furrows observed are represented in Text-figs. 4-8. The exine is smooth except for the occurrence of blunt spines which are sparsely dispersed all over the grains except at the furrows. A characteristic feature of the pollen grains is the occurrence of two sets of spines in definite rows on opposite sides of the germ-pore and along its longitudinal axis (Text-fig. 3).



Text-Figs. 1-8. *Costus speciosus*. Fig. 1. An uni-nucleate pollen grain ( $\times 940$ ). Fig. 2. Bi-nucleate pollen grain ( $\times 940$ ). Fig. 3. A mature pollen grain. Note the exine pattern and germ pore ( $\times 430$ ). Figs. 4-8. Outline drawings of pollen grains to show the various types of furrows ( $\times 430$ ).

(iii) *Prochromosomes*.—The diploid number of chromosomes in *Costus speciosus* is 36 (Pl. VI, Fig. 11). In the somatic nuclei a number of deep staining globular bodies lie irregularly distributed at prophase (Text-fig. 9). Some of these show the presence of slight constrictions and two of them are seen to be regularly attached to the nucleolus which takes up a peripheral position. The number of these dark staining bodies is 36 (Text-fig. 9). With the commencement of prophase these globular bodies—the prochromosomes—seem to elongate a little and most of them appear to be constricted (Text-fig. 10). At a slightly later stage the intertwined nature of the chromosomes is revealed (Text-fig. 11). The chromosomes do not show the presence of anastomoses and the nucleolus also maintains its spherical outline. The chromosomes next become fully organised and show their characteristic morphology. Two of the chromosomes are still seen to be attached to the nucleolus; of these one is slightly shorter than the other. The later stages of mitosis that follow show no irregularity in the process. In the late telophase stage when the daughter nuclei are organised, the chromosomes retain their morphology, but with the increase in size of the nuclei the length of the individual chromosomes appears to be greatly reduced and minute globular bodies as noted in the prophasic nuclei are once more evident. Thus it appears that these bodies which are commonly referred to as the prochromosomes are the portions of chromosomes which persist and pass on from the telophase to the succeeding prophase.

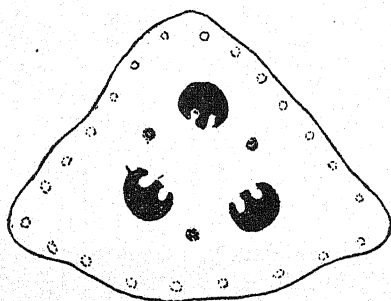


Text-Figs. 9-11. *Costus speciosus*. Fig. 9. Resting nucleus showing thirty-six prochromosomes, two of which are attached to the nucleolus ( $\times 1440$ ). Fig. 10. Constricted areas become prominent in the prochromosomes ( $\times 1440$ ). Fig. 11. Chromosomes have become organised ( $\times 1440$ ).

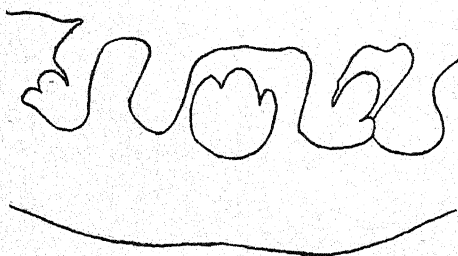
(iv) *Structure of the ovule*.—The gynæcium of *Costus speciosus* is tricarpeillary and the ovary trilocular (Text-fig. 12). The placenta is derived from the fused margins of the carpels and bears two to four rows of anatropous ovules. Each row consists of six to eight ovules.

The ovule at first arises as a papillate protrusion of the placental tissue. A single hypodermal archesporial cell becomes differentiated in the nucellus even before the curvature of the ovule primordium. The origin of the integumental tissue is next noted and the ovule gradually turns through an angle of 180 degrees and becomes anatropous in form. The direction of the curvature of the ovule is

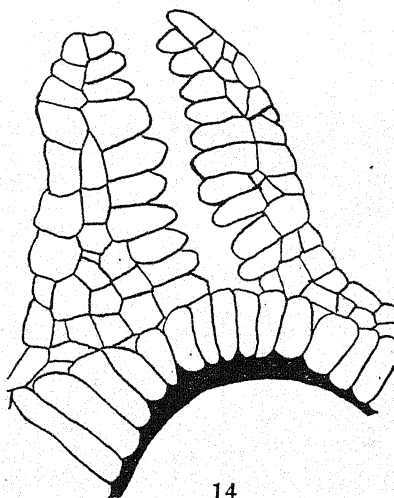
not the same in every instance (Text-fig. 13). The inner integument at its inception is composed of two layers of cells, but at a later stage of development the tip is seen to be composed of three layers. The innermost cells of this integument which line the micropyle grow out in a radial direction and assume an elongated form (Text-fig. 14). This seems to be a characteristic feature of the family Zingiberaceæ as it has been figured by Mauritzon<sup>9</sup> in the many forms he has studied. Gregory,<sup>4</sup> however, does not make any mention of it nor his figures indicate its presence. These cells lie closely adpressed in the mature ovule and probably play some part in the conduction of the pollen tube. The outer integument which develops simultaneously with the inner, is comparatively massive and is composed of four to five layers of cells. It takes no part in the formation of the micropyle. As noted previously by Humphrey<sup>6</sup> an aril is present, the development of which will be discussed later.



12



13



14

Text-Figs. 12-14. *Costus speciosus*. Fig. 12. Tricarpeillary ovary bearing two rows of ovules ( $\times 34$ ). Fig. 13. The direction of the curvature of the ovules ( $\times 94$ ). Fig. 14. The horizontal disposition of the innermost cells of the inner integument ( $\times 430$ ).

The nucellus in the initial stages of the development of the ovule is composed of a few layers of cells, but with the increase

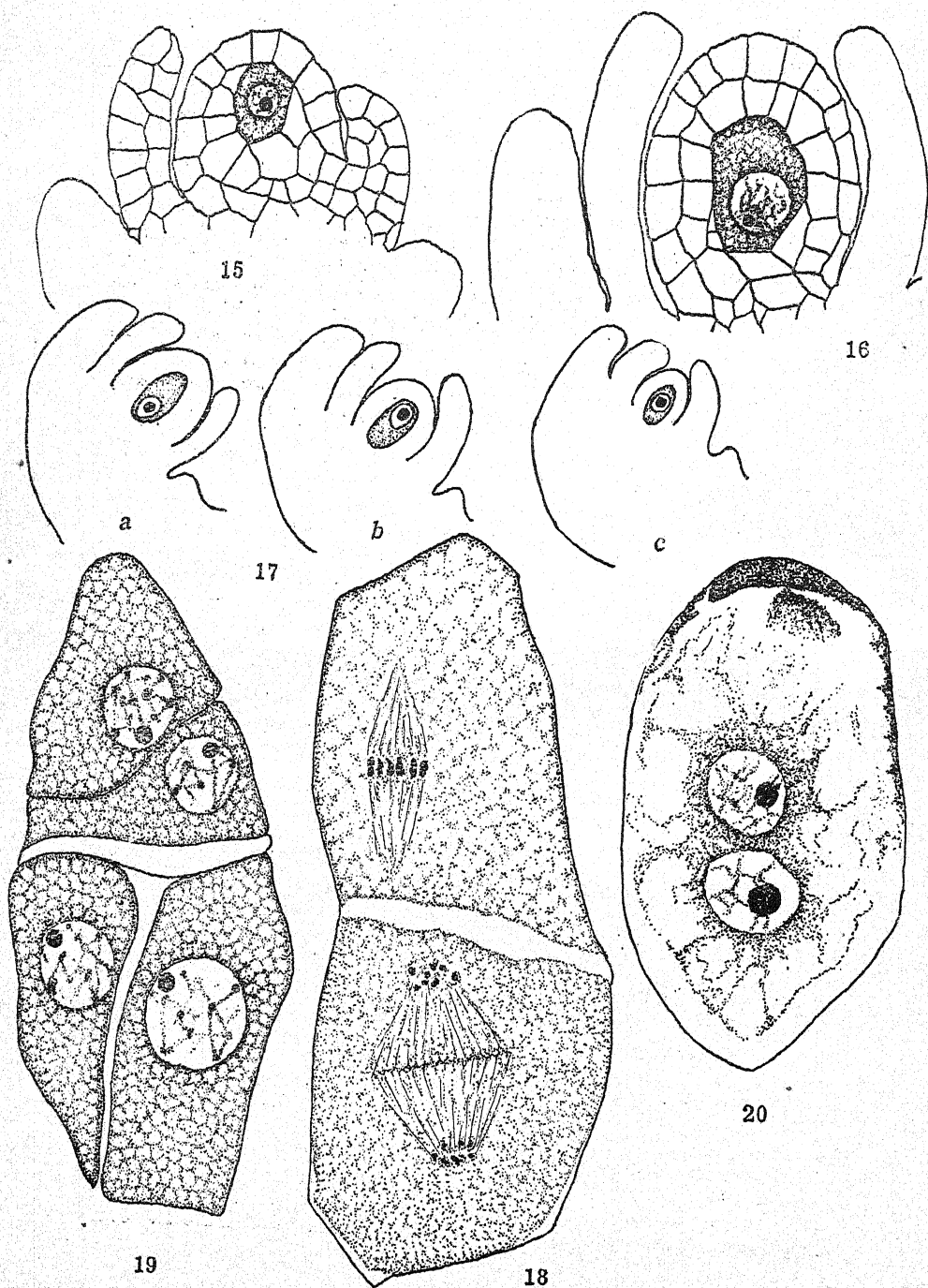


in size of the ovule a many-layered nucellus results. It is interesting to note in this connection that generally from the tetrad stage onwards the outer cells of the nucellus lying close to the micropyle show enhanced growth in a radial direction and become conspicuous. Further increase in length of these cells takes place after the post-fertilization stages. The occurrence of such radially elongated epidermal cells capping the nucellus seems to be another characteristic feature of the family Zingiberaceæ—nay that of the order Scitamineæ—as Mauritzon<sup>3</sup> has shown it to be present in *Strelitzia Regineæ*, *Heliconia aurantiaca*, *Stromanthe lutea*, *Calathea picturata* and in many other plants. It is curious that Gregory<sup>4</sup> does not make any mention of this nor does his drawings show these differentiated cells. A re-examination of the structure of the ovules of *Elettaria cardamomum* therefore appears to be necessary. As noted by Humphrey<sup>5</sup> a chalazal mass of tissue occurs at the base of the ovule. The cells of this tissue are smaller and are closely packed together and contain dense cytoplasm and nucleus.

(v) *Megasporogenesis*.—As has been stated before a single archesporial cell differentiates in the hypodermal layer of the nucellus and could be easily made out on account of its bigger size and greater chromaticity of the nucleus (Text-fig. 15). It cuts off a parietal cell and then functions as the megaspore mother-cell (Text-fig. 16). The parietal cell does not undergo any division but becomes somewhat elongated as the ovule increases in size. During the later stages of the development of the gametophyte this cell is crushed out by the growing embryo-sac which then comes to lie next to the radially elongated epidermal cells of the nucellus. This seems to be a general condition in all the species of *Costus*, as it has been noted in *C. cylindricus* by Boehm<sup>2</sup> and in *C. igneus* by Mauritzon.<sup>3</sup>

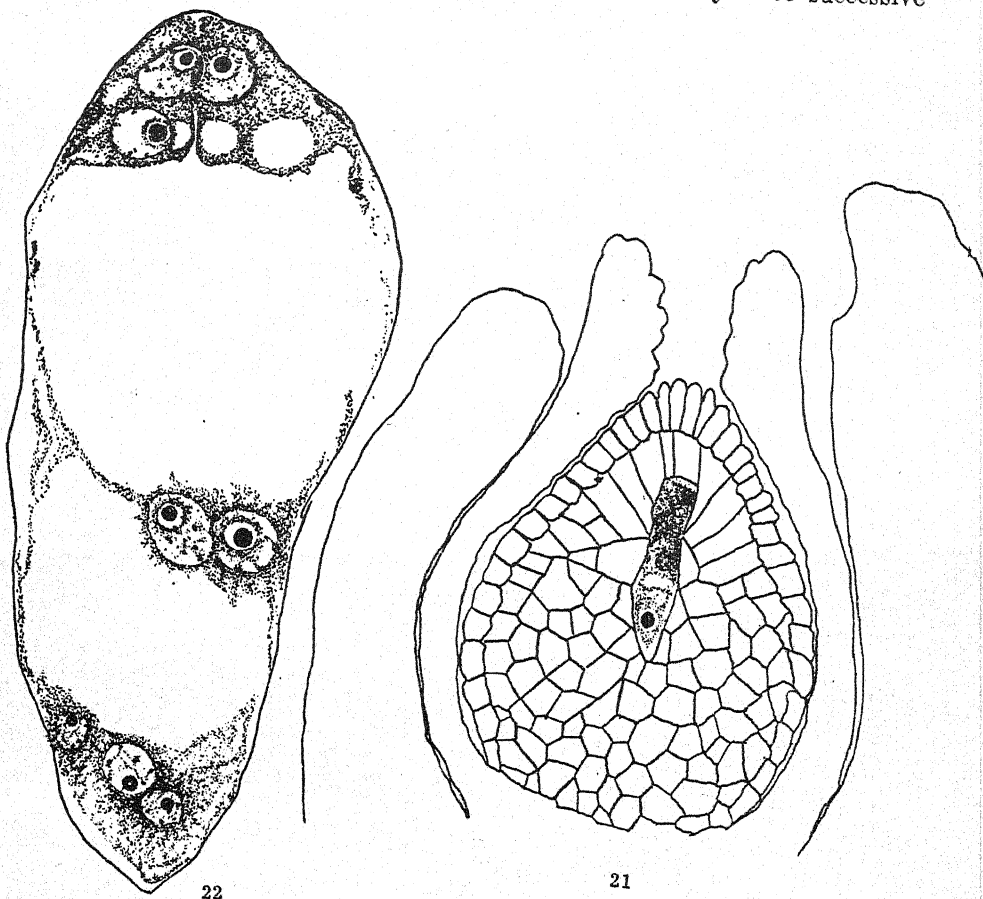
The megaspore mother-cell increases in size and becomes somewhat elongated before division I. At the early prophasic stages the nucleus seems to change its position inside the cell, as in some preparations the nucleus was observed to lie towards the base of the cell, in others towards the apex, and in some others it was centrally situated (Text-fig. 17 *a, b* and *c*). The heterotypic spindle is, however, oriented in the central region. The spindle fibres are well-developed and the chromosomes are aligned regularly at the equatorial region. On the completion of the division a phragmoplast develops which shows the presence of a series of fine granules in the central region as is commonly seen in cells in which cytokinesis takes place by cell plate formation. The two daughter nuclei formed as a result of division I, are separated by a distinct wall which is transversely disposed. The second division soon takes place (Text-fig. 18), and gives rise to a tetrad of megaspores (Text-fig. 19). The only variable feature is the arrangement of the megaspores resulting in different forms of tetrads. The proportion of linear tetrads as compared to the type represented in Fig. 19 is low. The upper three megaspores degenerate in every





Text-Figs. 15-20. *Cestus speciosus*. Fig. 15. The origin of the archesporial cell in the hypodermal layer of the nucellus ( $\times 940$ ). Fig. 16. The megaspore mother-cell ( $\times 940$ ). Fig. 17 a, b, c. Different positions.

instance and the lower one becomes functional. Fig. 21 represents a stage where the degenerating megaspores could be seen as dark shapeless masses capping the functional one. By three successive



Text-Figs. 21-22. *Costus speciosus*. Fig. 21. Degenerated megaspores capping the functional one ( $\times 430$ ). Fig. 22. A mature embryo-sac ( $\times 1440$ ).

mitotic divisions the chalazal megaspore produces an eight-nucleate embryo-sac in which two groups of four nuclei are seen at the two poles separated by a large central vacuole. Fig. 20 represents a bi-nucleate stage of the embryo-sac. The embryo-sac increases considerably in size from the bi-nucleate stage onwards and when

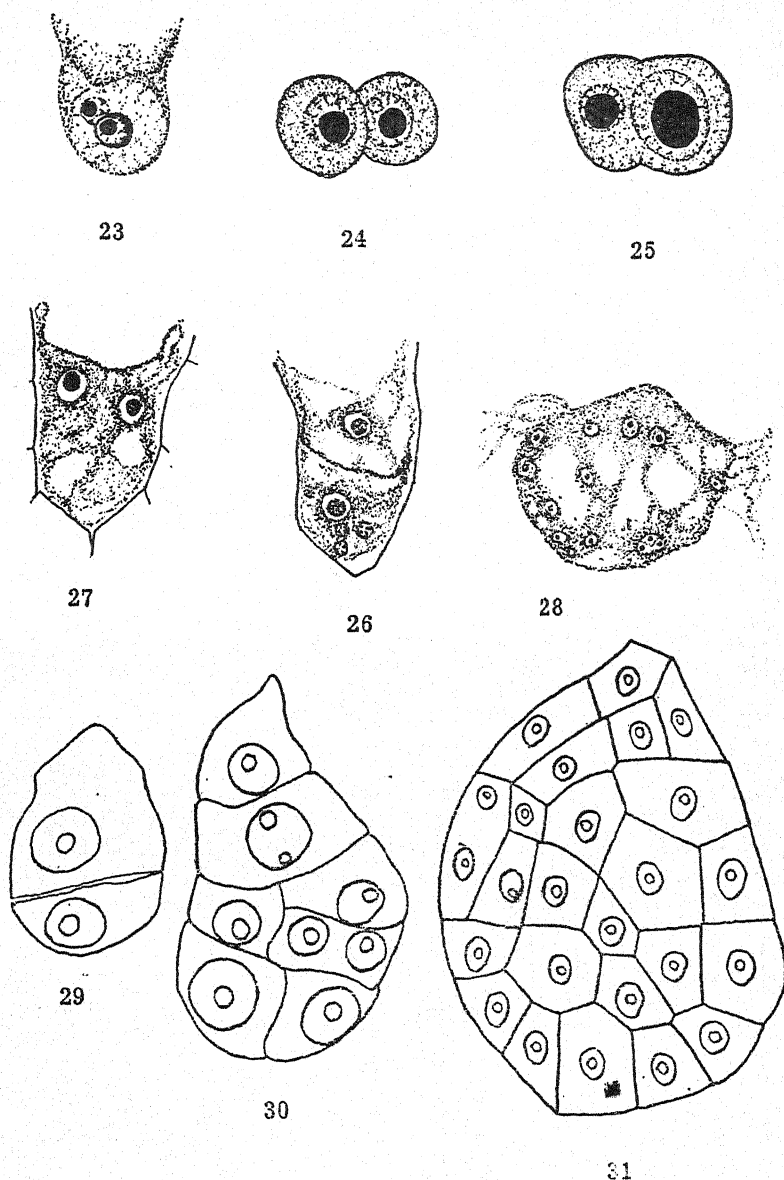
occupied by the nucleus of the megaspore mother-cell ( $\times 220$ ). Fig. 18. Homotypic division ( $\times 1550$ ). Fig. 19. Tetrad of megaspores ( $\times 1550$ ). Fig. 20. Bi-nucleate embryo-sac ( $\times 1550$ ).

fully developed lies just below the radially elongated epidermal cells of the nucellus.

The mature embryo-sac is encased by the two integuments and is somewhat cylindrical in shape. The synergids are pear-shaped in appearance and have big vacuoles at the base, the nuclei being situated above them. The egg shows the usual structure. Its position is below the synergids and is often masked by them. The polar nuclei at first lie close together but later fuse to form the secondary nucleus of the embryo-sac. The antipodals are three in number and lie in a mass of dense cytoplasm at the chalazal end of the embryo-sac (Text-fig. 22).

(vi) *Fertilization*.—The binucleate pollen grains on germination on the stigma traverse through the style and enter the ovule by way of the micropyle. This has been noticed in *Costus cylindricus* by Böhm.<sup>2</sup> The stages of division of the generative nucleus have not been observed but its form while inside the embryo-sac cavity is spherical. This would indicate that the generative nucleus divides while inside the pollen tube. Though several pollen tubes were seen at the top of the micropyle yet only one passed into it. Text-fig. 23 represents a stage where a plasmogamic condition of the egg and the generative nucleus is seen. It will be noted that the generative nucleus is smaller in size. Text-fig. 25 represents a stage of double fertilisation, in which the second male gamete which is smaller in size lies inside the cytoplasm of the secondary nucleus. Fusion of the polar nuclei is represented in Fig. 24 where the two nuclei are alike.

(vii) *Development of the endosperm and embryo*.—The primary endosperm nucleus migrates towards the chalazal region of the embryo-sac before division. At this stage deposition of pectic material takes place in the chalazal region of the embryo-sac as also below the epidermal cells of the nucellus (Text-fig. 14). Similar deposition of pectic material has also been observed by Böhm<sup>2</sup> in *Costus cylindricus*. The primary endosperm nucleus always divides prior to the division of the fertilized egg. The daughter nuclei become separated by a plasmatic membrane which divides the embryo-sac into a large micropylar and a small chalazal chamber (Text-fig. 26). The basal endosperm nucleus lies inside the chalazal chamber of the embryo-sac. The degenerated remnants of the antipodal cells are also seen lying close to the nucleus. The nucleus of the micropylar chamber moves upwards and undergoes many free nuclear divisions, while the nucleus of the chalazal chamber divides slowly and produces from 16–24 nuclei lying in a globular cytoplasmic mass (Text-fig. 28). The embryo-sac has been growing during this time and has increased considerably in length. At this stage the initial stages in the development of the embryo are noted. The later behaviour of the endosperm nuclei has not been followed on account of the difficulty of obtaining perfect sections of the seed. Humphrey,<sup>6</sup> however, states that "the endosperm of *Costus* and of the Zingiberaceæ, perhaps of all, reaches a considerable thickness,



Text-Figs. 23-31. *Costus speciosus*. Fig. 23. Plasmogamic condition of the egg and a generative nucleus ( $\times 1500$ ). Fig. 24. Fusion of the polar nuclei ( $\times 2200$ ). Fig. 25. Double fertilisation ( $\times 2200$ ). Fig. 26. Micropylar and basal chamber of the endosperm ( $\times 430$ ). Figs. 27-28. Bi-nucleate and multi-nucleate condition of the basal chamber ( $\times 430$ ). Figs. 29-31. Stages in the development of the embryo ( $\times 940$ ).

although it never wholly fills the cavity. In the ripe seeds of *Costus* it forms a single cell-layer over the micropylar end, and a layer several cells thick upon the wall of that part of the sac below the edge of the micropylar collar, more or less completely filling the space between the embryo and the wall of the cavity".

The fertilized egg rests for sometime before it starts activity. The first division of the oospore is transverse resulting in a large basal and a small terminal embryo cell (Text-fig. 29). The basal cell does not divide further and the later development of the embryo appears to depend on the activity of the terminal cell. Text-figs. 30 and 31 show different stages of the developing embryo. As observed by Humphrey,<sup>6</sup> the embryo is without a suspensor. The later stages of the development of the embryo have not been observed.

The observations made on the structure of the seed are essentially in accordance with the observations of Humphrey.<sup>6</sup> The aril develops from the upper part of the free end of the outer integument and the portion of the funicle lying close to the micropyle. The cells in these regions grow rapidly, coalesce and form a spongy mass which caps the micropylar end of the seed.

#### DISCUSSION

Mention has already been made about the chief features in the development of the ovule and the integuments. The mode of development of the female gametophyte, however, appears to be controversial. Humphrey<sup>6</sup> who has worked on *Costus* sp. states that "the mother-cell of the embryo-sac enlarges as the ovule grows, but does not divide further and thus becomes itself the definitive embryo-sac". As Humphrey's study was chiefly confined to the development of the ovule, seed and aril, it is likely that he had missed the earlier stages. Mauritzon also records a "*Lilium*-type" of embryo-sac development in *Costus igneus* and thus corroborates Humphrey's<sup>6</sup> observations. The present investigation, however, shows beyond doubt the normal type of embryo-sac development, as the various stages in the development of the macrospores have been observed. A preliminary note on the subject has already been published.<sup>1</sup> It thus becomes difficult to account for Mauritzon's<sup>9</sup> findings, and one is led to infer that probably more than one type of embryo-sac development occur in this family. Maheshwari<sup>7</sup> in his review of embryo-sac types in angiosperms records a number of plants, in which different types of embryo-sac development have been recorded by different investigators. It may however, be mentioned in this connection that the development of the female gametophyte in other plants of the family Zingiberaceae as recorded till now by different workers is of the normal type.

The development of the endosperm presents no unusual features and as recorded by previous workers is of the "*Helobiales*" type. Boehm<sup>2</sup> has observed the presence of four nuclei in a row in the basal endosperm of *Costus cylindricus*. No such arrangement has

been found in this material and it seems likely that the linear arrangement of the four nuclei is a chance phenomenon. The basal endosperm grows in size with the increase in the number of nuclei, some of which might be disposed in a linear direction. The plasma membrane which delimits the basal endosperm becomes thinner and thinner with its increase in size and it is probable that at a later stage the cytoplasm of the micropylar endosperm may become continuous. Definite evidence of this phenomenon was, however, not obtained.

The occurrence of nucleolar buds has been noted by different workers in various plants and there are different views on the subject. Tischler<sup>16</sup> is of opinion that nucleolar budding is an artefact due to fixation. Carlson and Stuart<sup>2</sup> state that the material composing these buds is of extraneous matter and is extruded by the cell. Mazumdar and Datta<sup>8</sup> state that these buds which get detached from the nucleolus contribute chromaticity to the developing spireme. In *Costus speciosus* minute globular buds have been noted to remain attached to the nucleolus during early prophase. Sometimes these get detached and lie close to the nucleolus or move towards the periphery, but in no case they were observed to lodge on the spireme. Further, in some nuclei these buds have been observed to grow in size and attain the same dimension as the primary nucleolus, and remain attached to it. At diakinesis, generally two nucleoli of equal size are seen which disappear almost simultaneously with the development of the metaphase spindle. It is interesting to note that Selim<sup>14</sup> working on rice found that in certain varieties the single large nucleolus of the pollen mother-cells budded out a secondary nucleolus which became as big as the mother nucleolus. He states that these two nucleoli are connected by a short bridge and show differences in staining reaction. The secondary nucleolus being responsible for the formation of the chromosomes while the primary nucleolus contributes in some way to the formation of the spindle. Nandi<sup>11</sup> has also observed budding of the nucleolus in rice and concludes as follows:—"Only the larger nucleoli bud and that these occur in the large nuclei. The budding moreover takes place after the nucleolar growth and means simply a transfer of material from the larger nucleolus to the smaller and not an increase in the total amount of the nucleolar material". Comparative measurements of a large number of nuclei of meiocytes of *Costus speciosus* seem to support this view. The growth of the nucleus brings about an increase in the nucleolar material which induces it to bud. Against this hypothesis it may be argued that the growth of the nucleus in the earlier stages of meiosis is of common occurrence but budding is not universal. Thus one is forced to the conclusion that budding of the nucleolus is due to the influence of fixing fluids and is an artefact.

The chromosome numbers of a few species of *Costus* has till now been determined. Böhm<sup>2</sup> reports the presence of eight chromosomes in the meiocytes of *Costus cylindricus*, while Gregory<sup>4</sup>

states that the diploid number of chromosomes in *Costus Malabariensis* is 18. In the present investigation 18 haploid and 36 diploid chromosomes have been observed in the meiotic and somatic nuclei respectively. It thus appears that *Costus speciosus* is a tetraploid-form. Similar instances of the occurrence of polyploid forms within a genus has been recorded in a number of plants and among the cultivated plants, cotton, wheat and rice could be cited as examples.

In concluding this paper mention should be made of the morphology of the pollen grains of *Costus* which presents some interesting features. The furrows, which are two in number, are of unequal size and form, and they show no definite space relationship on the exine. As very little is at present known of the exine pattern or pollen morphology of other plants of the family Zingiberaceae no comparative statement could be made. Gregory<sup>4</sup> states that the pollen grains of *Elettaria cardamomum* are spiny and believes it to be the only instance of spiny microspores in monocotyledons. It is interesting to note that the pollen grains of *Costus speciosus* also show the presence of blunt spinous processes.

#### SUMMARY

The paper gives an account of the cytology and embryology of *Costus speciosus*.

1. The formation of the microspores takes place by successive division and the meiotic process appears to be normal. Cytokinesis is by cell-plate method. The microspore tetrads show an isobilateral arrangement.
2. The diploid number of chromosomes is 36 and the haploid 18. It appears that the plant is a tetraploid.
3. The budding of the nucleolus as observed during the early meiotic process is described and an explanation has been offered to account for the phenomenon.
4. The pollen grains are bi-nucleate at the time of shedding. The generative cell is lenticular in shape and is separated by a cytoplasmic membrane.
5. The pollen grains show the presence of blunt spines which are irregularly distributed on the exine. Two sets of spines occur in definite rows on opposite sides of the single germ-pore and along its longitudinal axis. There are two furrows of which one is commonly branched.
6. The occurrence of pro-chromosomes in the somatic nuclei has been recorded. Their number corresponds to the diploid number of the plant. The behaviour of the chromosomes during the mitotic cycle lends support to the theory that pro-chromosomes are the portions of chromosomes which persist through the telophase.
7. The ovules are anatropous in form. The nucellar epidermis composed of radially elongated cells.



8. The archesporial cell arises in the hypodermal layer of the nucellus, it cuts off a parietal cell and then functions as the megaspore mother-cell. Due to increase in size of the embryo-sac during later stages of development, the parietal cell is disorganised and the embryo-sac lies just below the nucellar epidermis.

9. The megaspore mother-cell passes through the various stages of reduction division and produces a tetrad of megaspores of which the lower one becomes functional.

10. A normal eight-nucleate embryo-sac is produced which shows the usual organisation. The antipodals and the synergids degenerate later.

11. Fertilization is porogamous. The sperms appear as spherical nuclei.

12. The development of the endosperm is of the 'Helobiales' type. The basal apparatus forms a globular cytoplasmic mass which contains from 16-24 free nuclei.

13. The first division of the zygote is transverse. It develops into a cylindrical embryo which is without a suspensor.

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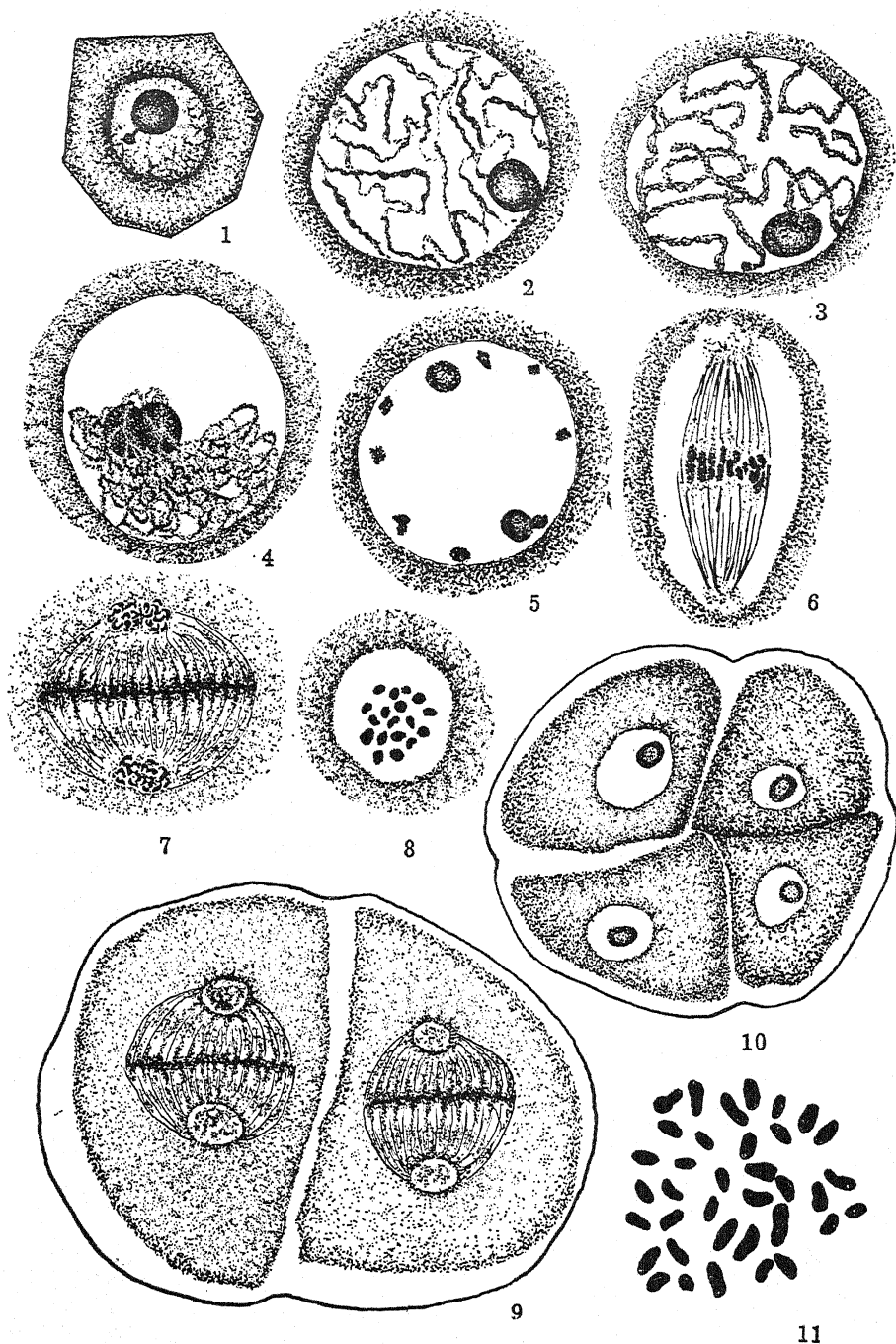


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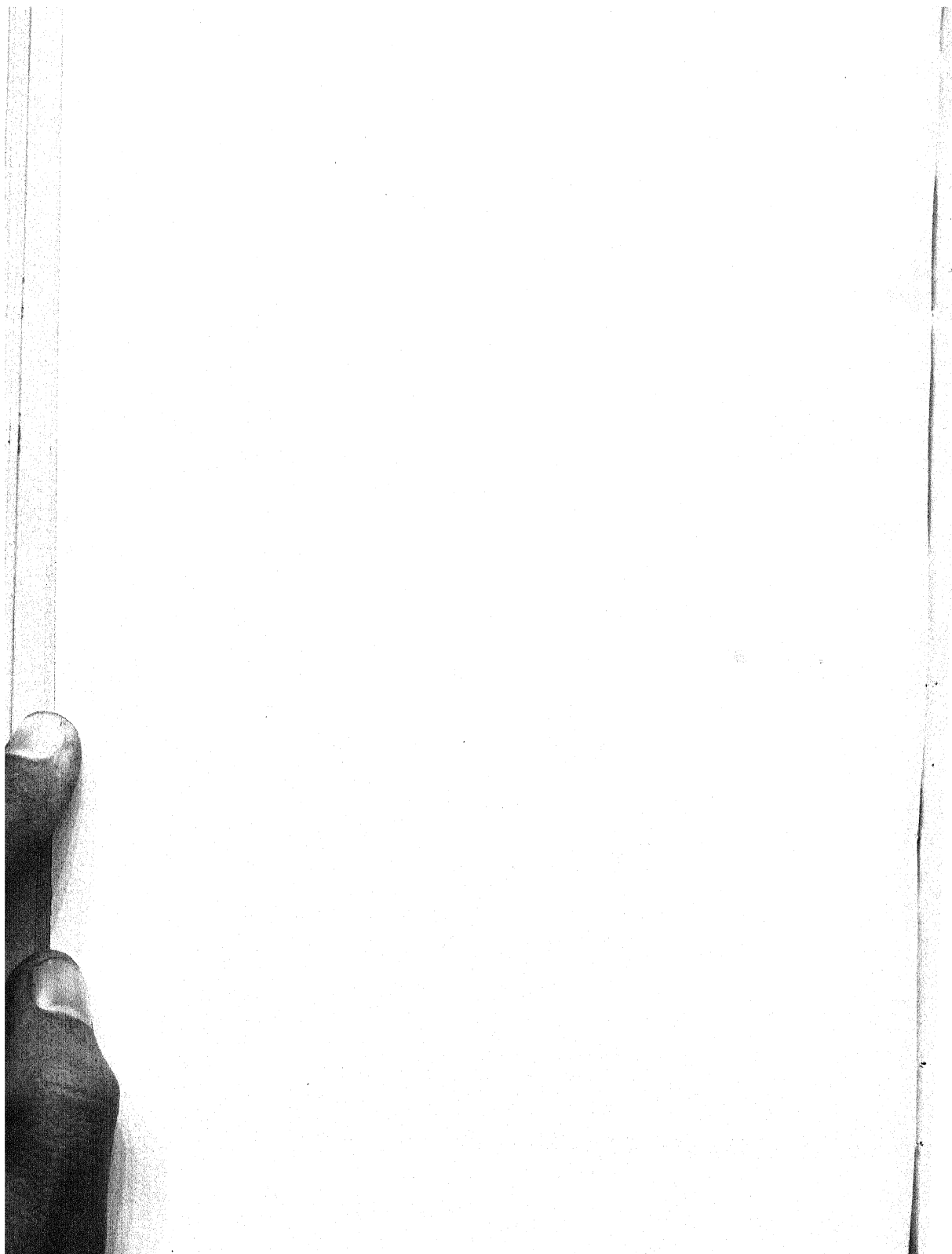
## EXPLANATION OF PLATE

All the figures were drawn with the aid of a camera lucida. Figs. 1-10 have been drawn at a magnification of  $\times 1440$ , Fig. 11  $\times 3650$ .

- FIG. 1. Resting nucleus showing a nucleolar bud.
- FIG. 2. Leptonema stage.
- FIG. 3. Zygonema stage.
- FIG. 4. Synizesis; note nucleolar bud.
- FIG. 5. Diakinesis; note the two nucleoli which have originated as a result of the budding of the single nucleolus.
- FIG. 6. Heterotypic metaphase.
- FIG. 7. Late anaphase; note cell plate formation in the centre.
- FIG. 8. Polar view of an equatorial plate (Div. I) showing 18 chromosomes.
- FIG. 9. Homotypic division; Telophase.
- FIG. 10. Isobilateral arrangement of pollen tetrad.
- FIG. 11. A somatic chromosome plate showing 36 chromosomes.



I. BANERJI—A CONTRIBUTION TO THE LIFE-HISTORY OF  
*COSTUS SPECIOSUS* SMITH



# MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN THE SCROPHULARIACEÆ

## \*II. Floral Morphology and Embryology of *Angelonia grandiflora* C. Morr. and Related Genera

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(Communicated by T. S. Raghavan)

Received for publication on August 24, 1940

	CONTENTS	PAGE
I.	INTRODUCTION .. .. .	198
II.	MATERIAL AND METHODS .. .. .	199
III.	<i>Angelonia grandiflora</i> C. Morr. .. .. .	199
	(a) The Ontogeny of the Flower .. .. .	199
	(b) The Development of the Female Gametophyte .. .. .	200
	(c) Embryo .. .. .	204
	(d) Endosperm .. .. .	204
	(e) Haustorium .. .. .	205
IV.	<i>Dopatrium lobelioides</i> Benth. .. .. .	205
	(a) Development of the Female Gametophyte .. .. .	205
	(b) Tapetum .. .. .	206
	(c) Development of Embryo-sac .. .. .	206
	(d) Endosperm .. .. .	207
	(e) Embryo .. .. .	210
V.	<i>Stemodia viscosa</i> Roxb. .. .. .	210
	(a) The Organogeny of the Flower .. .. .	211
	(b) Development of the Ovule .. .. .	211
	(c) Endosperm Haustoria .. .. .	212
	(d) Embryo .. .. .	212
	(e) The Mature Seed .. .. .	212
VI.	<i>Vandellia crustacea</i> Benth. .. .. .	213
	(a) Endosperm .. .. .	213
	(b) Embryo .. .. .	215
	(c) Tapetum .. .. .	215
VII.	DISCUSSION .. .. .	216
	(a) Persistent Synergids .. .. .	216
	(b) Endosperm Haustoria .. .. .	217
	(c) Integumentary Tapetum .. .. .	217
VIII.	SUMMARY .. .. .	218
IX.	ACKNOWLEDGEMENT .. .. .	219
X.	LITERATURE CITED .. .. .	219

\* Thesis (in part) approved for the degree of Master of Science of the Annamalai University.

## I. INTRODUCTION

BALICKA-IVANOWSKA in 1899 made one of the best contributions to our knowledge of the embryo-sac development in this family. She studied a number of families of the Sympetalæ, which included several genera of the Scrophulariaceæ. In particular, the question of nutrition in the embryo-sac has been dealt with. The haustoria were always formed in contact with parts well supplied with nutrition, so that it was believed that the haustoria conducted nourishment into the embryo-sac. Balicka-Ivanowska (1899) considered that the tapetal cells served to pass nutritive substances into the embryo-sac and that it possibly had a digestive function. In 1906, Schmid investigated numerous species of the Scrophulariaceæ and discussed the formation of the embryo-sac, fertilization, endosperm formation and the development of the haustoria. In 1915, Mitchell investigated the embryo-sac and embryo of *Striga lutea*. Evans (1919) has given a review of the work done in the Scrophularineæ upto the time of Mitchell (1915) and has described the haustoria and the embryo in *Pentstemon secundiflorus*. Schertz (1919) found the order of development of the floral parts to be calyx, stamens, corolla and pistil and also describes the endosperm haustorium and the development of the embryo. Cook (1924) gave an account of the development of the seed in *Linaria vulgaris*.

Warren (1924) described hybrids obtained between different species of *Digitalis*. Buxton and Newton (1928) gave the chromosome numbers of many plants of this family, and described interspecific crosses in *Digitalis*. Krishna Iyengar (1929, 31, 33, 34) described the embryo-sac and the endosperm haustorium in *Ilysanthes*, *Bonnaya*, *Sopubia delphinifolia*, and *Alonsoa*, in all of which, the endosperm nucleus gives rise to a row of three cells, of which the two towards the chalazal and micropylar ends of the embryo-sac develop into the respective haustoria, while the middle cell gives rise to the endosperm tissue. Srinath (1934) in a short note described the life-history of *Herpestis monniera*. Krishna Iyengar, in a later paper (1939a) described the embryo-sac and the formation of the endosperm haustorium in *Celsia coromandeliana* and *Isoplexis canariensis*. In both the above members, he found a compact tissue composed of radiating cells and abutting on the chalazal end of the embryo-sac and the endosperm haustorium in these consists of four chalazal and four micropylar cells. Srinath (1938) in a short note described the endosperm haustorium in *Calceolaria*. Krishna Iyengar (1939b) in another short note reports that in *Stemodia viscosa*, the chalazal part of the embryo-sac is enlarged, while the micropylar part is tapering. In *Vandellia hirsuta* and *Vandellia scabra*, persistent antipodals were reported by the same author and in *Limnophila heterophylla* and *Stemodia viscosa* he has found a single large and highly aggressive uninucleate chalazal haustorium and in *Centranthera* he has figured what are called secondary micropylar haustoria. Krishna Iyengar (1939c) in a later paper describes in detail the embryo-sac and the development of the endosperm haustorium in *Limnophila* and *Stemodia*.

## II. MATERIAL AND METHODS

The following genera were investigated:—*Angelonia*, *Dopatrium*, *Stemodia* and *Vandellia*. Most of the materials for the present work were collected from plants grown in the University Botanical Gardens, Annamalainagar. *Dopatrium* was collected from the rice fields, where it grows in abundance and *Vandellia* from the edges of ponds and streams nearby. Ovaries and ovules were fixed either in Formalin Acetic Alcohol or in hot corrosive sublimate fixative. For studying the organogeny of the flower, floral tips were fixed in Formalin Acetic Alcohol. Mature seeds were soaked in glacial acetic acid for a number of days before fixation, in order to facilitate sectioning. Materials were dehydrated in alcohol, cleared in chloroform, and embedded in paraffin. Sections were cut at thicknesses varying from 6 to 14 microns. All the preparations were stained with Haidenhein's iron alum hæmatoxylin.

III. *Angelonia grandiflora* C. Morr.

*Angelonia* is not an indigenous plant. It is an exotic genus being a native of South America (Bailey, 1933). This is grown in the gardens for the ornamental flowers, which are present throughout the year. The plant produces a large number of minute triangular seeds, but propagation is by vegetative means, on account of the ease with which cuttings produce roots. *Angelonia grandiflora* is a herbaceous perennial with the main stem somewhat woody and possessing a strong aromatic odour. The whole plant is covered by numerous glandular hairs. Flowers are axillary, and two flowers invariably arise in the axil of each leaf. The flowers are borne on a long pedicel. The corolla consists of two lips, the posterior lip being composed of two lobes and the anterior of three lobes. The two lips are fused in such a way as to form a boat-shaped structure, the posterior end of which is connected to the thalamus while in the anterior end there is present a nectary. There are four fertile anthers.

*The Ontogeny of the Flower.*—The primordium of the flower arises as a knob-like protuberance in the axil of a leaf (Fig. 1). The two floral primordia in each axil are seen in Fig. 1 (1 and 2). The order of development of the floral parts was found to be calyx, stamens, corolla and carpels. Schertz (1919) found the same order of development of the floral organs in *Scrophularia marylandica*. The primordium of the calyx arises from the sides of the apical dome as a fold in the outer layer (Fig. 1 se). The anterior sepals are the first to arise (Fig. 1). This is brought about by the repeated anticlinal divisions of the outer layer at this region. Some of the cells of the second layer below this region divide periclinally and as a result the sepal primordium increases in size (Fig. 2). The next floral organ to arise is the stamen and not the petal (Fig. 1 st). The primordium of the stamen also arises from the sides of the dome. The mode of formation of the stamen initials is exactly the same as that for the sepal. Soon after

the stamens begin to enlarge, the primordia of the petals are initiated slightly below the point of origin of the stamen (Fig. 1 at pe). Periclinal divisions of the second layer of cells in this region occur, and the petal initials grow in size (Fig. 3). The two carpels (Fig. 4, ca) arise from the central domed apex, after the other floral organs have been initiated. A careful examination of this region shows that the two carpels arise not from the terminus of the central domed apex that remains, but distinctly from the sides of the dome (Fig. 4). Thus the carpels are found to have a lateral origin and this has been utilized by Newman (1936) and Raghavan (1937) to support the classical foliar concept of the carpels.

*The Development of the Female Gametophyte.*—The ovary is of the typical bilocular Scrophularious type. The numerous ovules arise as small protuberances of the axile placenta. The hypodermal archesporial cell is soon differentiated and differs from the surrounding cells in its larger size, larger nucleus, and richer cell contents (Fig. 5). Sometimes, there are two archesporial cells, either juxtaposed (Figs. 6 and 7) or superposed (Fig. 8). The archesporium is differentiated in the ovule even before the initiation of the integument (Fig. 5). Such an early differentiation of the primary archesporium is a common feature not only in the Scrophulariaceae, but also in many others. For example, Joshi and Rao (1934) have reported such an early differentiation in *Digera*. It also seems to be common in the Juglandaceae, where Langdon (1935) has reported it in *Carya* and *Juglans* and Woodroff (1928) in *Hicoria pecan*. And in the Orobanchaceae, Srivastava (1939) has recorded it in *Orobanche aegyptiaca*. Multicellular archesporia have also been reported in various genera distributed over a wider range of families. Bhaduri (1935), who has investigated a number of species of *Solanum* in which multicellular archesporia have been found, is of the opinion that they are derived from the transverse or longitudinal divisions of the initial hypodermal archesporium. So far as could be seen in the present investigation, no evidence could be found to regard the 2-archesporial stage as a derived condition. There is no evidence of division of an initially single archesporium. From the earliest stage, the two cells can be distinguished individually. By the time the archesporium is differentiated in the ovules, the anthers show the pollen tetrad stage. This shows the decisive protandry of the plant.

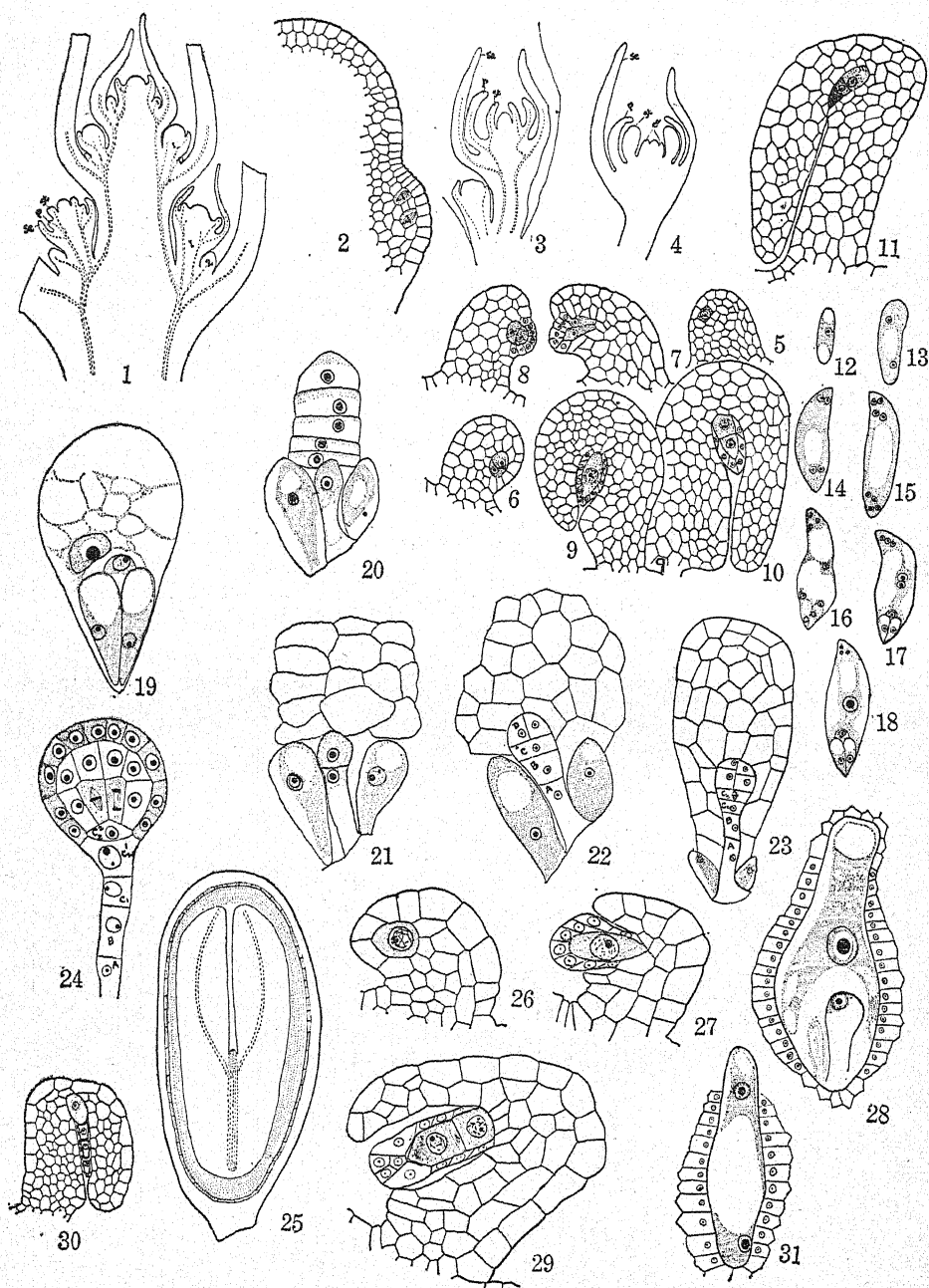
One of the archesporial cells develops and directly functions as the megaspore mother-cell (Fig. 9). By this time, the single massive integument grows rapidly and the anatropous nature of the ovules is revealed. The bulk of the ovule at this stage is made up of the integument (Fig. 9). The megaspore mother-cell, as soon as it is formed is invested almost to its base by a layer of cells, which is regarded as the nucellus. There is no parietal tissue, the hypodermal archesporium functioning directly as the megaspore mother-cell, without cutting off any primary wall-cell. It seems likely, that this single layer of what is called the nucellus, is primarily

derived from the original domed apex, which caps the archesporial cell (Fig. 7). The cells of the nucellus stain very lightly compared to the rest of the ovule. With the development of the megaspore mother-cell, the cells of the nucellus get flattened, and finally disorganise and completely disappear in the early stages of the development of the embryo-sac. Such reduced nucellus consisting of a single layer of cells, which disorganise during the early stages of the development of the embryo-sac, seems to be a characteristic feature of the Scrophulariaceæ and a few other families. Balicka-Ivanowska (1899) calls it "nucelle" and reports its occurrence in *Pedicularis palustris* (Scrophulariaceæ), *Klugia notoniana* (Gesneraceæ), *Campanula rotundifolia* (Campanulaceæ) and others. It is also common in the Solanaceæ, where Bhaduri (1935) has figured the nucellar tissue in a number of Solanaceous plants. It is also present in the Lobeliaceæ, in *Lobelia nicotianefolia* (Kausik, 1938) and in the Orobanchææ, in *Orobanche aegyptiaca* (Srivastava, 1939) and also in many other members of the Rubiaceæ. Schertz (1919) says that in (*Scrophularia marylandica*, the nucellus is destroyed towards the micropylar part of the ovule, due to the elongation of the embryo-sac, the nucellus remaining intact in the chalazal part and that the final disappearance of the nucellus takes place, while the endosperm is being formed. I find, however, that the nucellus degenerates earlier, during the initial stages of the development of the embryo-sac.

In the majority of Scrophularious plants, a layer of tapetal cells is invariably formed as a band of cells surrounding the embryo-sac. The details of the formation of the tapetum in the genus *Dopatrium* are described later. In *Angelonia* no tapetal layer is developed. Mitchell (1915) reports the absence of any tapetal layer in *Striga lutea*.

The megaspore mother-cell undergoes the heterotypic division giving rise to a dyad (Fig. 10). The dyad undergoes the homotypic division and forms a linear tetrad of megaspores (Fig. 11). The one towards the chalazal end develops into the embryo-sac, while the rest degenerate. The functioning megaspore increases in size and there appear vacuoles on either side of the more or less centrally situated nucleus (Fig. 12). In the bi-nucleate embryo-sac, there are two nuclei, one at each end of the embryo-sac, with a big vacuole between them (Fig. 13). Another division of the two nuclei gives rise to the four-nucleate stage (Fig. 14). Between the four-nucleate stage and eight-nucleate stage, there is not much increase in the size of the embryo-sac (Fig. 15). The two polar nuclei move towards the centre of the embryo-sac. Occasionally, the polar nucleus from the chalazal end reaches the centre of the embryo-sac, earlier than the polar nucleus from the micropylar end (Fig. 16). At this time, the organisation of the synergids is vaguely visible. The two polar nuclei move towards the centre of the embryo-sac and fuse at about the middle part of the embryo-sac (Fig. 18). Schmid (1906) has pointed out that the place in the embryo-sac, where the polar nuclei meet is not constant, and instanced





Figs. 1-25. *Angelonia grandiflora*. Fig. 1. Floral tip. Note the two flowers (1 and 2) arising in each axil. Note the primordia of the stamen. Note the origin of the petal, after the stamen primordium has been initiated.  $\times 150$ . Fig. 2. Origin of the sepal primordium by the periclinal division

*Pedicularis palustris*, where he found the polar nuclei fusing in the upper, lower and middle part of the embryo-sac.

The formation of the mature eight-nucleate embryo-sac would thus appear to conform to the normal monosporic type (Maheshwari, 1937). Five divisions intervene between the megaspore mother-cell and the eight-nucleate embryo-sac. The mature embryo-sac (Fig. 17) consists of two synergids, an egg-cell, two polar nuclei, and three antipodals. The synergids are prominent each having at its base a large vacuole, above which the nucleus is placed.

The antipodals are ephemeral and degenerate soon after fertilization. The behaviour of the synergids after fertilization is interesting. They do not degenerate after fertilization, as in the case of the other members of this family, but persist till comparatively late stages in the development of the embryo. The normal function of the synergids is to direct the male gamete towards the egg nucleus and help in their fusion, and as soon as this fusion has

of the hypodermal layer of cells.  $\times 1500$ . Fig. 3. The sepals (se), the petals (p) and the stamens (st) and the domed apex.  $\times 750$ . Fig. 4. Shows the lateral origin of the carpels (ca).  $\times 750$ . Fig. 5. The early differentiation of the hypodermal archesporium.  $\times 750$ . Figs. 6 and 7. Two juxtaposed archesporial cells.  $\times 750$ . Fig. 8. Two superposed archesporial cells.  $\times 750$ . Fig. 9. The megaspore mother-cell and the single layer of nucellar tissue surrounding it.  $\times 750$ . Fig. 10. The dyad of megaspores with the enveloping nucellus.  $\times 750$ . Fig. 11. Linear tetrad, the chalazal one is the functioning megaspore.  $\times 750$ . Fig. 12. Uni-nucleate embryo-sac. Two large vacuoles, one on either side of the centrally situated nucleus.  $\times 750$ . Fig. 13. Binucleate embryo-sac. The two nuclei are separated by a large vacuole.  $\times 750$ . Fig. 14. Four-nucleate embryo-sac.  $\times 750$ . Fig. 15. 8-Nucleate embryo-sac, showing two groups of four nuclei, one at each end.  $\times 750$ . Fig. 16. Shows the early migration of the chalazal polar nucleus.  $\times 750$ . Fig. 17. The two polar nuclei meeting at about the middle of the embryo-sac.  $\times 750$ . Fig. 18. The two polar nuclei have fused.  $\times 750$ . Fig. 19. Shows the embryo-sac in a post-fertilization ovule, which has increased considerably in size. The synergids are persistent. The fusion nucleus has migrated towards the oospore.  $\times 1500$ . Fig. 20. The two persistent synergids and the oospore. Note the vacuole in the synergid cell. The endosperm nucleus has divided to form a row of cells.  $\times 1100$ . Fig. 21. Two celled embryo with the two persistent synergids one on either side. The endosperm tissue has increased.  $\times 1100$ . Fig. 22. The quadrant. The four cells have been designated A B C and D. The persistent synergids are still seen.  $\times 1100$ . Fig. 23. The octant with the persistent synergids. The cell C has divided transversely into  $C_1$  and  $C_2$ . The lower cell  $C_2$  is seen undergoing another transverse division.  $\times 750$ . Fig. 24. The primary meristems have been differentiated. The cell  $C_2$  has divided transversely to give rise to  $C_2^1$  and  $C_2^2$ . The cell  $C_1^1$  is the hypophysis. The dermatogen and the plerome are shaded.  $\times 1280$ . Fig. 25. Shows a section of the mature seed. The plumule is seen as a papillate protuberance between the cotyledons.  $\times 355$ . Figs. 26-38. *Dopatrium lobelioides*. Fig. 38. Two hypodermal archesporial cells.  $\times 1500$ . Fig. 26. An archesporial cell functioning directly as the megaspore mother-cell.  $\times 1500$ . Fig. 27. Megaspore mother-cell invested by a single nucellus.  $\times 1500$ . Fig. 29. Shows the dyad enveloped by the nucellus.  $\times 1500$ . Fig. 30. Tetrad of megaspores. The chalazal one is the functioning megaspore.  $\times 750$ . Fig. 31. Two-nucleate embryo-sac, with a large vacuole separating the two nuclei.  $\times 1500$ .

taken place, the synergids usually degenerate. In some plants, as for example, *Celsia coromandeliana* and *Isoplexis canariensis* (Krishna Iyengar, 1939a), one of the synergids is destroyed, when the pollen tube enters the embryo-sac and reaches the egg-cell, the other degenerating soon after. The synergids in *Angelonia* do not degenerate after fertilization, but persist long afterwards (Figs. 19-23). The synergids are seen clearly in post-fertilization ovules, which have increased in size considerably (Fig. 19). In the synergids, the basal vacuoles are clearly seen and above them the nuclei are present.

*Embryo*.—The fertilized egg undergoes a period of rest and increases in size to some extent. Above the oospore nucleus is a large vacuole. The persistent synergids can be seen one on either side of the oospore (Figs. 19 and 20). The nuclei of the synergids can be seen above a terminal vacuole. The first division of the oospore is transverse (Fig. 21). The two cells (Fig. 21) undergo another division transversely giving rise to a four-celled linear pro-embryo. For purposes of description, these four cells of the pro-embryo beginning from the uppermost, will be designated as A, B, C and D. Each one of these four cells gives rise to a definite region in the mature embryo proper. A longitudinal wall laid down in cell D produces a quadrant (Fig. 22). A transverse wall across the quadrants forms the octant stage (Fig. 23). The cell C divides transversely giving rise to two cells  $C_1$  and  $C_2$ , of which the lowermost  $C_2$ , functions as the hypophysis (Fig. 23). The cells A and B and also  $C_1$ , constitute the suspensor. The suspensor is thus three cells long. The embryo at the octant stage is six cells long. Fig. 24 shows a later stage of the embryo. The primary meristems dermatogen, periblem, and the plerome have been differentiated. The hypophysis  $C_2$ , has divided transversely into  $C_1^2$  and  $C_2^2$  (Fig. 23) the upper of the resulting two cells, becomes continuous with the dermatogen, while the lower  $C_2^2$ , forms part of the periblem (Fig. 24).

The mature seed (Fig. 25) consists of a large more or less oblong embryo with fairly long and equal cotyledons. The hypocotyledonary portion including the radicle and the cotyledons are more or less equal in length. Cook (1924) reports that in *Linaria vulgaris*, the mature seed consists of a very long embryo, with short unequal cotyledons. In the mature seed, the two plerome strands of the two cotyledons meet that of the root in the hypo-cotyledonary region. Between the cotyledons, is enclosed the plumule which looks like a small papillate protuberance. The embryo is embedded in a mass of endosperm cells, which is surrounded by a single layer of prominent cells, with thick cell walls. This layer of cells has a protective function. All these are surrounded by a seed-coat consisting of large thin-walled cells.

*Endosperm*.—The development of the endosperm has always been an interesting feature of the Scrophulariaceæ. Wall formation from the very first division of the primary endosperm nucleus is almost the rule in this family. In *Angelonia* cellular endosperm

is found. The primary endosperm nucleus moves from the middle part of the embryo-sac and comes to lie close to the egg (Fig. 19). There, after undergoing a period of rest, it divides a number of times to form a single row of cells (Fig. 20). Divisions in planes perpendicular to the original soon set in and serve to increase the endosperm tissue (Fig. 21).

*Haustorium*.—Endosperm haustoria are a characteristic feature of the Scrophulariaceæ. They have been reported in almost all the members worked out so far, including those which exhibit free-nuclear division of the endosperm nucleus. Two kinds of endosperm haustoria usually occur, the micropylar and chalazal. In some cases, one of them is present, in other cases both kinds of haustoria are met with. With regard to their number, shape, size, structure, the number of nuclei present in the haustorial cell, and the degree of their aggressiveness, much variation has been found but their presence in some form or other has always been a constant feature. In *Angelonia*, however, no endosperm haustorium, either micropylar or chalazal is noticeable. Schertze (1919) reports that no haustoria are to be found in *Scoparia*, but reports instead the presence of a tissue composed of many cells in the chalazal end of the ovule. It is supposed to pass on nutritive material to the embryo and the endosperm.

#### IV. *Dopatrium lobelioides* Benth.

This is a small marshplant which grows in profusion in the rice fields, soon after the rains. The plant grows to a height of about 18 inches. There is a group of leaves at the base. Above, the leaves are very much reduced. Flowers are borne terminally, and are pedicelled. The corolla is bilabiate, the posterior lip consisting of two lobes and the anterior consists of three lobes. There are two fertile stamens and two undeveloped stamens.

*Development of the Female Gametophyte*.—The ovary in *Dopatrium* is different from the majority of Scrophularious plants. Bilocular ovary and axile placenta are the characteristic features of a large number of members of this family. In *Dopatrium* the ovary is uni-locular and bicarpellary, and the numerous anatropous ovules are arranged on both sides of two parietal placentæ. Even before the initiation of the integument, the primary archesporium is differentiated as a hypodermal cell and is more prominent than the surrounding cells (Fig. 26). Fig. 38 shows a plate of two archesporial cells. The primary archesporium increases in size, elongates and directly functions as the megaspore mother-cell (Fig. 27). Just at the time when the archesporium increases in size and assumes the role of the megaspore mother-cell, the primordium of the single integument appears as an annular outgrowth from the base of the ovule and grows down towards the placenta very rapidly. The megaspore mother-cell as soon as it is formed is surrounded by a single layer of nucellar tissue. This single layer of nucellar cells is short lived for it soon becomes caught as it were, between the enlarging megaspore mother-cell and the integument and is finally crushed.

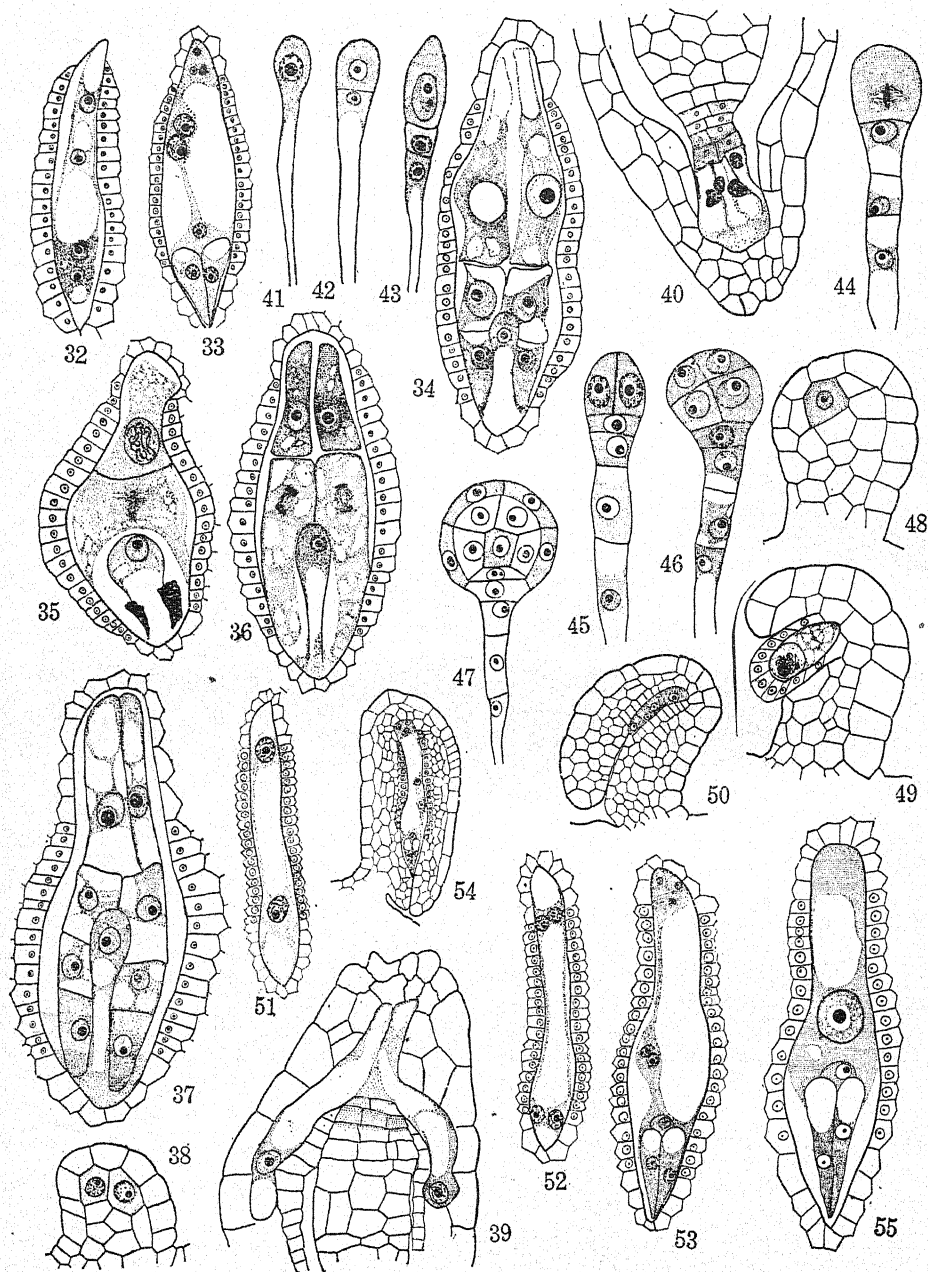
*Tapetum*.—As a result of the breakdown of this single layer of nucellar tissue, investing the megaspore mother-cell, the innermost layer of the integument comes into direct contact with the sides of the embryo-sac. This layer of cells becomes conspicuous, on account of the regularly arranged rectangular cells, and they soon come to possess rich cell contents. This differentiation commences simultaneously from the micropylar and chalazal ends of the embryo-sac. This single layer of cells differentiated from the integument appears as a row or band of cells on either side of embryo-sac (Fig. 31). This layer of cells is the tapetum and has a nutritive function. A similar method of origin of the tapetum has been recorded by Cooper (1931) in *Lycopersicum esculentum*. The tapetum does not surround the embryo-sac completely. At the chalazal and micropylar ends of the embryo-sac, only ordinary cells are found (Fig. 31). In *Vandellia scabra*, *Vandellia hirsuta* (Krishna Iyengar, 1939b) and in *Vandellia crustacea* described later in this paper, the active tapetal layer is confined to the chalazal half of the embryo-sac, the tapetum ending abruptly at the micropylar end, though Schmid (1906) found that the tapetum might cover all the embryo-sac in certain cases. In *Stemodia viscosa*, Krishna Iyengar (1939c) found the tapetum covering the entire embryo-sac except the chalazal end, though my own observations on the same plant described later differ from his. The cells of the tapetum are roughly rectangular in shape and lie with their long axis perpendicular to the embryo-sac. They contain dense cytoplasm, and a single prominent nucleus and as the tapetal cells grow in size, a vacuole is formed. Svensson (1926) has reported the occurrence of starch kernels in the tapetal cells of *Hyoscyamus niger*. The tapetal cells covering the embryo-sac are usually uninucleate, though binucleate tapetal cells are of common occurrence as in *Solanum melongena* (Bhaduri, 1932), and in *Orobanche aegyptiaca* (Srivastava, 1939). A distinct tapetal tissue surrounding the embryo-sac has been reported in the Solanaceae (Bhaduri, 1935), in the Labiateae (Billings, 1909), in the Lobeliaceae (Kausik, 1938) and in the Orobanchae (Srivastava, 1939).

*Development of Embryo-sac*.—At the stage when the megaspore mother-cell is about to enter upon the first reduction division, the ovule consists of an elongated megaspore mother-cell, surrounded by a single layer of nucellus. The bulk of the ovule at about this stage consists of the integument. As a result of the first reduction division, a dyad of megaspores are formed (Fig. 29). The dyad by another division gives rise to a linear tetrad of megaspores (Fig. 30). The one towards the chalazal end persists, while the remaining three megaspores degenerate. The functioning megaspore increases considerably in size, before its nucleus divides to give rise to an ordinary binucleate embryo-sac (Fig. 31). Figure 32 represents the four-nucleate embryo-sac. The eight-nucleate embryo-sac is derived in the usual manner. The mature embryo-sac is roughly spindle-shaped except for the fact that there is a bulging of the embryo-sac near the micropylar end (Fig. 33). The

eight-nucleate embryo-sac consists of two prominent synergids, an egg-cell, two large sized polar nuclei, and three antipodal cells. Each of the synergids, has at its base a large vacuole above which the nucleus is placed. In the egg cell towards the base is the nucleus, and above it there is a large vacuole. The polar nuclei are the largest nuclei in the embryo-sac (Fig. 33). Large sized polar nuclei of the kind occurring here, have been reported in a number of plants belonging to various families and in *Scrophularia marylandica* Schertz (1919) found the diameter of the polar nuclei to be twice that of either the egg, or the synergids. The two polar nuclei meet in the middle part of the embryo-sac and fuse. Soon after fertilization, the antipodals as also the synergids degenerate (Fig. 35).

*Endosperm.*—In the post-fertilization embryo-sac, the triple fusion nucleus is the most prominent and largest nucleus in the embryo-sac and it lies more or less in the middle of the embryo-sac (Fig. 34). The fusion nucleus undergoes a period of rest after which it divides. It divides long before the division of the oospore. The first division of the fusion nucleus is transverse, the division being followed by wall formation, as a result of which the embryo-sac is divided into two large, superimposed endosperm cells (Fig. 35). The cell which undergoes the next division, is the micropylar one. It undergoes a first longitudinal division (Fig. 35) followed by wall formation and as a result three endosperm cells are formed in the embryo-sac. The chalazal endosperm cell now undergoes a longitudinal division followed by septation. The number of endosperm cells becomes four (Fig. 36). The two chalazal cells without undergoing any further division whatsoever, begin to function directly as the two chalazal haustoria and show signs of their haustorial nature. The two micropylar endosperm cells undergo another longitudinal division, at right angles to the first division. As a result, two tiers of two cells each are formed. These two tiers of cells undergo a transverse division giving rise to two tiers of four cells each (Fig. 34). The two tiers of cells towards the micropylar end (Fig. 34) undergo another transverse division to form on the whole, two tiers of six cells each (Fig. 37). The middle tier of four cells, by repeated division, develops into the endosperm tissue, while the micropylar tier of two cells produces the four-nucleate micropylar haustorium. A few layers of the cells of the endosperm tissue towards the chalazal and micropylar haustoria are smaller in size than the remaining cells of the endosperm tissue and have richer cell contents and take a deeper stain (Figs. 39 and 40). These modified endosperm cells do the function of conduction of food materials instead of the usual one of storage. This kind of modification of some of the endosperm cells is intended to pass on the nutritive substances absorbed by the two types of haustoria, to the developing embryo and endosperm. Such modified endosperm cells have been recorded in *Sopubia delphinifolia*, *Alonsoa* sp., *Isoplexis canariensis*, *Celsia coromandeliana* and others (Krishna Iyengar, 1937, 1939a).





*Dopatrium lobelioides*. Fig. 32. Four-nucleate embryo-sac.  $\times 1500$ .  
 Fig. 33. Eight-nucleate embryo-sac. Note the large-sized polar nuclei.  
 The tapetum does not cover the embryo-sac at the chalazal and micropylar

The two chalazal haustorial cells elongate and reach more or less the chalazal end of the ovule. Their nuclei are large and prominent and lie more or less in the middle of the cells. Soon after the elongation of the chalazal haustorium towards the chalazal end has come to a stop, the two chalazal haustoria give rise to small protuberances on the side nearer the epidermis of the ovule. This protuberance grows rapidly sideways towards the epidermis of the ovule at first then down towards the micropylar end (Fig. 39). The nuclei of the haustoria migrate towards the protuberance and continue to remain at the tip of the growing haustorial protuberance. These two branches of the chalazal haustoria grow along the integument, one on either side of the embryo-sac and parallel to the tapetum towards the micropyle (Fig. 39). They were seen to reach nearly the middle of the ovule. A similar case has been found in *Rhamphicarpa longiflora* (Krishna Iyengar, 1939b), where the tetranucleate micropylar haustorium eats its way through the integument and reaches very nearly the chalazal haustorial tube. A layer of cytoplasm always surrounds the nucleus of the haustoria. The nuclei during this stage are not hypertrophied. Food materials are absorbed from the cells of the integument by these two chalazal haustoria and passed on to the endosperm. The chalazal haustoria are thus very aggressive and display a great degree of haustorial efficiency. The micropylar haustorium which is four-nucleate is also aggressive. It absorbs nutritive material from the micropylar end of the ovule. Compared with the chalazal haustoria, the

ends of the embryo-sac.  $\times 1500$ . Fig. 28. Post-fertilization embryo-sac. The triple fusion nucleus and the oospore are seen.  $\times 1500$ . Fig. 35. The endosperm nucleus has divided into two cells. The micropylar cell is undergoing a longitudinal division.  $\times 1500$ . Fig. 36. The chalazal endosperm cell has also divided longitudinally. The two cells function as the chalazal haustoria. The two micropylar cells (the two tiers of cells are not shown in the figure) undergo a transverse division to give rise to four cells. (Two tiers of four cells each) (see Fig. 34).  $\times 1500$ . Fig. 37. The two cells towards the micropylar end undergo a transverse division. Thus six micropylar cells (two tiers of six cells each) are formed. The oospore with its upper portion elongated is surrounded by the endosperm cells.  $\times 1500$ . Figs. 39–47: *Dopatrium lobelioides*. Fig. 39. The two highly aggressive chalazal haustoria. They have grown down towards the micropylar end. Only the outermost layer of the integument is present.  $\times 750$ . Fig. 40. Four-nucleate micropylar haustorium. Note the modified endosperm cells towards the micropylar haustorium.  $\times 750$ . Fig. 41. The elongated oospore.  $\times 1500$ . Fig. 42. Two-celled embryo.  $\times 1500$ . Fig. 43. Three-celled embryo.  $\times 1500$ . Fig. 44. Four-celled linear proembryo.  $\times 1500$ . Fig. 45. Quadrant.  $\times 1500$ . Fig. 46. Octant.  $\times 1500$ . Fig. 47. Shows the primary meristems. The dermatogen and the plerome are shaded.  $\times 1500$ . Figs. 48–55: *Stemodia viscosa*. Fig. 48. Hypodermal archesporium.  $\times 1500$ . Fig. 49. Megaspore mother-cell, surrounded by a layer of nucellus.  $\times 1500$ . Fig. 50. The linear tetrad. The chalazal megaspore is the functioning one.  $\times 750$ . Fig. 51. Bi-nucleate embryo-sac.  $\times 1500$ . Fig. 52. Four-nucleate embryo-sac.  $\times 1500$ . Fig. 53. Eight-nucleate embryo-sac.  $\times 1500$ . Fig. 54. Shows the position of the Eight-nucleate embryo-sac in the ovule. The tapetum is not present at the chalazal and micropylar ends of the embryo-sac.  $\times 750$ . Fig. 55. Note the large endosperm nucleus and the long synergids.  $\times 1500$ .



micropylar haustoria are less aggressive. In the later stages, the nuclei of the micropylar haustorium get hypertrophied.

*Embryo*.—After fertilization, the oospore undergoes a period of rest, usually till a considerable amount of endosperm tissue has been formed. The upper portion of the oospore now undergoes extensive elongation, without cell formation. The oospore is thus brought down right into the endosperm tissue, which is developing rapidly in the central portion of the embryo-sac and this is characteristic of most of the members of the Scrophulariaceæ, in which micropylar haustoria have been recorded. The embryo-sac at this stage can be differentiated into at least two portions; an upper or micropylar portion containing in the earlier stages the egg apparatus, and in the later stages, the upper portion of the elongated suspensor and the micropylar haustorium and a lower or chalazal part in which the endosperm tissue and the embryo proper develop. A similar condition is to be found in most of the members of the Scrophulariaceæ, and in various members of the Labiatae, reported by Billings (1909). In the early stages, the activity of embryo formation is very small compared to the development of endosperm tissue. This is also a characteristic feature of this family and has been recorded in *Isoplexis canariensis* and *Celsia coromandeliana* and others (Krishna Iyengar, 1939a).

The first division of the oospore (Fig. 41) is by a transverse wall (Fig. 42). Of the two resulting cells, the lower one gives rise to the embryo proper and the upper one to the suspensor. Fig. 44 shows the four-celled linear proembryo. The regions in the mature embryo, to which these four cells give rise, are the same as described before, for *Angelonia*. The arrangement of the cells in the four-celled proembryo may be in either of two ways; either they may be linear, as in the present case, or, the lower two cells may lie side by side, in which case the four-celled proembryo would be three cells long. The latter type of arrangement is found in families like Crucifereæ, Ranunculaceæ (Souges, 1913, 1919), Capparidaceæ (Raghavan, 1937) while the former arrangement is characteristic of the Rubiaceæ, Solanaceæ (Souges, 1922, 1924), Leguminosæ (Cooper, 1933) and in the Orobanchæ (Srivastava, 1939). A longitudinal wall formed in the lowermost cell of the four-celled proembryo gives rise to the quadrant stage (Fig. 45). A transverse wall across the quadrants, results in the octant stage (Fig. 46). Fig. 47 shows a later stage, in which the primary meristems have been differentiated. The mature embryo is essentially similar to that found in *Angelonia* and *Stemodia*.

#### V. *Stemodia viscosa* Roxb.

*Stemodia viscosa* is a highly branching herb and grows profusely in waste places. The leaves are opposite and they clasp the stem. Flowers are borne in the axils of leaves and are stalked. There are four fertile stamens. The mature embryo-sac and the development of the endosperm in this plant have recently been described by Krishna Iyengar (1939c). My own observations with regard to these points differ from his.

*The Organogeny of the Flower.*—The floral organs do not arise in acropetal succession. The order of development of the floral organs was the same as that found in *Angelonia* namely, calyx, stamens, corolla, and lastly the carpels. The exact mode of origin of the primordium of the various floral organs is essentially the same as that described in *Angelonia*.

*Development of the Ovule.*—The ovule is typically Scrophularious, being bicarpellary with numerous anatropous ovules on an axile placenta as found in *Angelonia*. The archesporium is differentiated as a hypodermal cell very early in the ovules, even before primordium of the single the integument has originated. The archesporium directly functions as the megaspore mother-cell without cutting of any parietal cell. The archesporium (Fig. 48) increases in size and becomes the megaspore mother-cell (Fig. 49), which is soon surrounded by a single layer of nucellar cells. Just as the time, when the archesporium begins to function as the megaspore mother-cell, the primordium of the single integument appears and grows down towards the placenta. The development of the integument is very rapid. As it grows, the anatropous nature of the ovules is assumed. The integument when fully formed is about 3 to 4 cells in thickness, but soon becomes 5 cells thick by the division of the cells of the integument (Fig. 54). The megaspore mother-cell elongates considerably prior to the reduction division. Fig. 50 shows the tetrad of megaspores. The chalazal one is the functioning megaspore, while the three micropylar ones degenerate. The development of the eight-nucleate embryo-sac is quite normal and the various stages leading up to it are shown in Figs. 51 to 53. Just when the single layer of nucellus investing the megaspores show signs of degeneration, the innermost layer of the integument becomes differentiated into a tapetum surrounding the embryo-sac. This integumentary tapetum, as in many other members of this family, does not surround the embryo-sac completely. At the micropylar and the chalazal ends of the embryo-sac, only ordinary cells are found (Figs. 51 to 53). Krishna Iyengar (1939c), however, reports that the tapetum sheathes the non-dilated micropylar portion also. The tapetal cells are of the usual rectangular shape and are uninucleate. The mature embryo-sac is rather deeply placed in the ovule, so that there are at the most only two layers of cells between the chalazal end of the embryo-sac and the epidermis, while between the micropylar end of the embryo-sac and the outer epidermis, there are about three to four layers of cells. Krishna Iyengar's figures, however, suggest that there are about seven intervening layers of cells. In the mature embryo-sac the chalazal end is rather broad, while the micropylar end is tapering. Krishna Iyengar also found the micropylar end to be rather tapering. He, however, reports that the chalazal part of the embryo-sac is very much enlarged, even before fertilization, and that there is an accumulation of starch grains in that region, and suggests that it may have a haustorial function even before it is separated by a cross wall during the division of the endosperm nucleus. I have not

found any such enlargement of the chalazal end of the embryo-sac before or even after fertilization.

*Endosperm Haustoria.*—The first division of the fusion nucleus is transverse, resulting in the formation of two superimposed cells in the embryo-sac (Figs. 56 and 57). The micropylar cell then divides longitudinally (Fig. 57). The chalazal cell also divides longitudinally. The resulting two cells assume the role of chalazal haustoria without any further division (Fig. 58). The two micropylar cells (Fig. 58) again divide longitudinally, at right angles to the first division, so that two tiers of two cells each are formed (Fig. 58). Two transverse walls laid down across these two tiers of cells produce two tiers of six cells each (Figs. 59 and 60). The two micropylar haustorial cells curve round the oospore, which in the meantime grows down towards the endosperm. Thus ultimately the micropylar haustorial cells come to lie side by side beyond the oospore. These endosperm cells towards the micropylar end form the four-nucleate micropylar haustorium (Fig. 61). The four tiers of cells in the middle of the embryo-sac by repeated divisions give rise to the endosperm tissue proper. Krishna Iyengar also found a four-nucleate micropylar haustorium. When the two types of haustoria have just been laid, the integument is about five cells thick. As the growth of the endosperm tissue proceeds, the integument decreases in thickness. This is due to the fact, that the tapetum is constantly digesting and absorbing the contents of the integumental layer close to it and passing it on to the developing endosperm tissue. This process of absorption of food materials from the cells of the integument by the tapetum and its subsequent transference to the endosperm tissue continues, and when the endosperm tissue is fully formed, there is left but a single layer of cells namely the outermost layer of the integument (Fig. 61). The tapetum thus serves a double purpose, absorption of food materials from the integumentary region and its transference to the endosperm tissue. In addition to the tapetum, the two types of haustoria also absorb nutrition from the two ends of the ovule.

*Embryo.*—The oospore undergoes a long period of rest during which time it elongates considerably, so that its lower end comes to lie in the midst of the endosperm tissue, which has been formed in the meantime. The oospore, as in the case of *Dopatrium*, begins to divide only after a considerable amount of endosperm tissue has been formed. The development is similar to that described for *Dopatrium*.

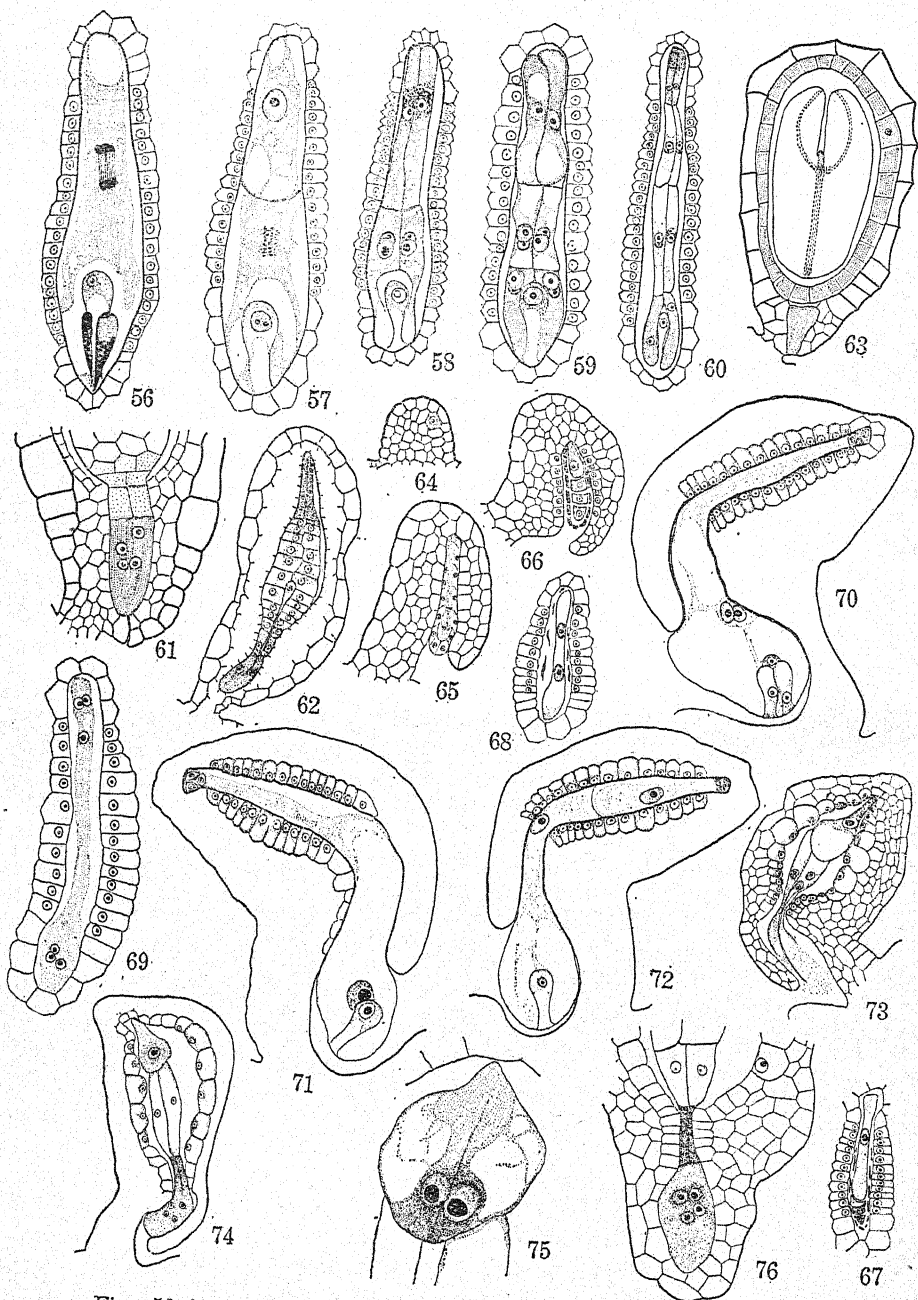
*The Mature Seed.*—It consists of a roughly oblong embryo with rather short and equal cotyledons (Fig. 63). A small amount of endosperm tissue surrounds the embryo. This is surrounded by a single layer of thick-walled cells. The seed-coat consists of a single layer of thin-walled cells.

VI. *Vandellia crustacea* Benth.

*Vandellia* is a small highly branching prostrate marshy herb. Flowers arise singly in the axils of leaves and are pedicelled. The corolla is bi-labiate, consisting of two posterior lobes and three anterior lobes. There are four fertile stamens. The ovary is bilocular with numerous anatropous ovules.

The development of the ovule and the embryo-sac is quite normal and is similar to those described in the case of *Angelonia*, *Dopatrium*, and *Stemodia*. As usual, a hypodermal archesporial cell is formed, which directly functions as the megaspore mother-cell (Fig. 64). There is a layer of nucellar cells surrounding the megaspore mother-cell (Fig. 65). A single integument is formed as usual. The megaspore mother-cell is rather longer in *Vandellia* than in the other members described before (Fig. 65). Fig. 66 represents the linear tetrad of megaspores. The chalazal one is the functioning one (Fig. 67). Fig. 68 shows the binucleate stage. All the four nuclei of the four-nucleate embryo-sac do not divide simultaneously. One of the two nuclei at each pole of the four-nucleate embryo-sac divides earlier than the other. As a result, there is a six-nucleate embryo-sac stage (Fig. 69). The micropylar portion of the embryo-sac is very much enlarged and the integument does not surround this swollen micropylar end of the embryo-sac. Such extra-ovular embryo-sac seems to be a common feature of the genus *Vandellia*, and has been reported by Krishna Iyengar (1939b) in two species of *Vandellia*, *hirsuta* and *scabra*. The enlarged micropylar end of the embryo-sac is pressed against the placenta and the funicle and probably absorb food materials directly from them. The chalazal half of the embryo-sac is bent and is somewhat long and narrow (Fig. 70). The embryo-sac is straight till the six-nucleate stage and the bending takes place after the six-nucleate stage. The integumentary tapetum encloses only the bent chalazal half of the embryo-sac. The cells of the tapetum are similar to those described for the previous genera and are uninucleate. In the eight-nucleate embryo-sac, there are two synergids, and egg nucleus, and three antipodals. The polar nuclei meet near the bend of the embryo-sac, and fuse (Fig. 70). The synergids degenerate after fertilization. The antipodals, on the other hand, are persistent and they can be seen even till after the division of the endosperm nucleus (Figs. 71 and 72).

*Endosperm*.—The fusion endosperm nucleus, after a period of rest, divides transversely. The division is followed by wall formation, resulting in two endosperm cells, the chalazal and micropylar (Fig. 72). The latter divides twice longitudinally as in the case of *Dopatrium* and *Stemodia* to produce two tiers of two cells each (Fig. 73). These two tiers of cells divide transversely this time, and thus two tiers of four cells each are formed (Fig. 74). The endosperm cells towards the micropylar end develop into a four-nucleate micropylar haustorium. The cross-wall disappears and the haustorium is ultimately one chambered



Figs. 56-63. *Stemodia viscosa*. Fig. 56. The endosperm nucleus dividing transversely.  $\times 1500$ . Fig. 57. Shows the chalazal and micropylar endosperm cells. The micropylar cell is seen dividing longitudinally.  $\times 1500$ . Fig. 58. Note the two chalazal haustorial cells and the two

(Fig. 76). The chalazal haustorial cell undergoes a longitudinal division and the resulting two cells become the chalazal haustorial cells (Fig. 75). These two chalazal cells often fuse as also their nuclei. As a result, in older chalazal haustoria only one nucleus is to be found (Fig. 74). The two tiers of endosperm cells in the middle of the embryo-sac give rise by repeated division to the endosperm tissue.

*Embryo*.—Some of the stages in the development of the embryo have been observed and they are essentially similar to that described for the previous members.

*Tapetum*.—The tapetum as has already been stated, surrounds only the bent and the narrow chalazal half of the embryo-sac (Fig. 70). The cells are of the usual rectangular shape and are more or less equal in size. The single nucleus of the tapetal cells is situated in that end of the cell, which is close to embryo-sac and in the opposite end of the cell, is a large vacuole. This kind of arrangement of the nuclei and the vacuole in a uniform manner is to be found only in *Vandellia* (Fig. 70). After a considerable amount of endosperm tissue has been formed, and the two kinds of haustoria have been laid down, a few, particularly two or three cells of the tapetum increase considerably in size and in older vacuoles, they are many times bigger in size than they originally were (Figs. 73 and 74). These cells of the tapetum absorb nutrition directly from the cells of the integument and pass it on to the endosperm tissue. By this process of transmission of the contents of the cells of the integument, to the developing endosperm, the whole of the integumentary tissue is eaten up and in older ovules, only the epidermis of the integument is left.

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tiers of micropylar cells.  $\times 1200$ . Fig. 59. Shows the two chalazal haustoria and the two tiers of four micropylar cells.  $\times 1200$ . Fig. 60. A later stage showing two tiers of six micropylar cells and the two chalazal haustorial cells.  $\times 700$ . Fig. 61. Four-nucleate micropylar haustorium.  $\times 750$ . Fig. 62. Shows the relative positions of the chalazal and micropylar haustoria, and the endosperm tissue and the embryo.  $\times 355$ . Fig. 63. Mature embryo.  $\times 355$ . Figs. 64-76. *Vandellia crustacea*. Fig. 64. Hypodermal archesporium.  $\times 750$ . Fig. 65. Megaspore mother-cell surrounded by the nucellus.  $\times 750$ . Fig. 66. Linear tetrad. The functioning megaspore is the chalazal one. The single layer of nucellar tissue shows signs of degeneration.  $\times 750$ . Fig. 67. The innermost layer of the integument is differentiated into the tapetum.  $\times 750$ . Fig. 68. Bi-nucleate embryo-sac. Note the remains of the degenerated nucellus and the integumentary tapetum.  $\times 750$ . Fig. 69. Six-nucleate embryo-sac.  $\times 1200$ . Fig. 70. Eight-nucleate embryo-sac. Note the bent and the extra ovular embryo-sac. The tapetum is confined to the chalazal half of the embryo-sac.  $\times 750$ . Fig. 71. The endosperm nucleus situated close to the oospore. Note the persistent antipodals.  $\times 750$ . Fig. 72. The endosperm nucleus has divided into two cells.  $\times 750$ . Fig. 73. The micropylar cell has divided longitudinally into two cells. Some of the tapetal cells have enlarged considerably.  $\times 355$ . Fig. 74. Shows the uninucleate chalazal haustorium. Four-nucleate micropylar haustorium and the endosperm.  $\times 355$ . Fig. 75. The binucleate chalazal haustorium, the nuclei about to fuse.  $\times 1500$ . Fig. 76. Four-nucleate micropylar haustorium.  $\times 750$ .



## VII. DISCUSSION

*Persistent Synergids.*—Maheshwari (1937) observes "Abnormalities concerning the synergids are much rarer. Rodolico (1930) reports that they are specially prominent in *Buphthalmum salicifolium* and Pætow (1931) writes that in *Dysosyllum ramifolia* they reach almost to the middle of the embryo-sac". Persistent synergids have also been reported in a few other plants belonging chiefly to the Compositæ and Berberidaceæ. In this connection, it may be mentioned, that the Small (1919) has recorded the occurrence of antipodal haustoria in *Campanula americana*, *Campanula rotundifolia*, and *Lobelia inflata*. Mauritzon (1936) has observed the persistence of one of the synergids in *Berberis vulgaris*, *B. cretica*, *B. empetrifolia* and in *Mahonia aquifolium*. He observes, in this connection, "Aller Wahrscheinlichkeit nach ist es diejenige Synergide, die nicht vom Pollenschlauch durchdrungen wird, die sich weiter entwickelt, während die andere schnell degeneriert". In these plants, one of the synergids persists and assumes a haustorial character. Hegelmair (1886) and Eichinger (1907) have reported that in *Chrysosplenium alternifolium*, the synergids may persist and become hypertrophied. Gaumann (1919) has on the other hand proved that this is on account of a mistake on the part of the authors. He says they are really suspensor cells, and have been mistaken for synergids. Dahlgren (1924) and Schurhoff (1926) have studied the genus *Calendula* and have found that a large synergid haustorium is developed. Dahlgren (1924) has also reported a similar synergid haustorium in *Ursinia anthemoides*. In this connection he says, "Genau wie bei *Calendula* entwickelt sich ein Synergidenhaustorium. Es verlängert sich stark und frisst sich bis zum Funikulus vor, was aus der in Fig. 4 reproduzierten Mikrophotographie hervortgeht". The synergid haustorium in this plant grows towards the micropylar end of the ovule and reaches very near the funicle. This work of Dahlgren was afterwards confirmed by Schurhoff (1926).

In *Angelonia*, the two synergids persist and even increase in size to some extent, but they do not develop any haustorial protuberance similar to that found in *Ursinia anthemoides*. It cannot be said definitely whether they perform any haustorial function, without developing any haustorial protuberance, such as is the case in *Berberis* and *Mahonia* (Mauritzon, 1936). But their persistence taken in conjunction with the absence of any endosperm haustorium, either chalazal or micropylar, seems to be of some significance. Because, endosperm haustoria have always been a very characteristic feature of the Scrophulariaceæ and wherever endosperm haustoria were reported, their function has always been one of supplying nutrition to the embryo and the endosperm. The absence of any kind of endosperm haustoria and the presence of persistent synergid instead, seems to be of some significance. It may not be improbable that these synergids, are haustorial in function, replacing to some extent the endosperm haustoria, which are a feature of almost universal occurrence in this family. So far as is known, the only

other genus of this family, where haustoria do not occur is *Scoparia* (Schertz, 1919). Unfortunately, however, the work in question is not of such a detailed character as to throw any light upon this aspect. This genus is now being investigated to find out whether the haustoria are really absent and if so, whether this is correlated with the persistence of the synergids, such as has been found in the genus *Angelonia*.

*Endosperm Haustoria*.—The method of formation of the endosperm haustoria in the genera investigated seems to be more or less similar to one another, but different from cases already reported. In the majority of the genera a row of three cells is formed of which the middle produces endosperm tissue, while the other two give rise to the micropylar and chalazal haustoria respectively. These, however, vary in form and in the number of cells composing them. For example, while in *Alonsoa*, *Isoplexis* and *Celsia* (Krishna Iyengar, 1937, 1939a) this three-cell method is adhered to, variation is to be found in the make-up of the haustoria. In all the three genera, the micropylar haustorium is four-nucleate, and the chalazal haustorium in *Alonsoa* consists of two uni-nucleate cells, while that in *Celsia* and *Isoplexis* are four-nucleate. In other genera, different methods of formation of the haustoria have been observed. In *Bonnaya* (Krishna Iyengar, 1931) the fusion nucleus divides once transversely resulting in the formation of two cells. The chalazal one divides longitudinally, the resulting two cells are organised into the chalazal haustorium, and the micropylar cell, by a series of divisions, gives rise to four simple unbranched vermiform micropylar haustoria, and endosperm cells towards the interior. In *Striga lutea* (Mitchell, 1915) a transverse division of the endosperm nucleus occurs and two cells are formed. The nucleus of the chalazal cell divides once without septation, and this bi-nucleate cell becomes the chalazal haustorium. The micropylar cell divides repeatedly each division followed by wall formation. No definite micropylar haustorium is present. In the present investigation, the general method followed is different from any of those mentioned above. The first division is transverse resulting in the formation of two superimposed cells. The micropylar cell divides twice longitudinally in two planes at right angles to each other, to produce two tiers of two cells each. The chalazal cell also divides longitudinally, the resulting two cells become the chalazal haustoria. The two tiers of micropylar cells form two tiers of six cells each as a result of the formation of two transverse cross-walls. The two tiers of cells towards the micropylar end produce a four-nucleate haustorium, while the remaining tiers of cells in the middle of the embryo-sac produce the endosperm tissue. In *Vandellia*, however, there is but one transverse division of the two tiers of micropylar cells.

*Integumentary Tapetum*.—Compared to the constant presence of the tapetal tissue in the micro-sporangium, the occurrence of a tapetal layer surrounding the embryo-sac, is only rarely to be met with. In the microsporangia, the tapetum, which



is closely adpressed to the microspore mother-cells, is bounded on the outside by three or four layers of wall cells. Generally in the pollen sac, the innermost parietal layer functions as the tapetum. In the genera investigated, no parietal tissue as such is organised, the hypodermal archesporium functioning directly as the megaspore mother-cell, without cutting off primary wall cell. This, however, is made good by the single massive integument the innermost layer of which differentiates as the tapetum, which ultimately comes to envelop the embryo-sac, the intervening nucellus having degenerated. This takes place, when the linear tetrad is being organised. The contents of the cells of the tapetum in the microsporangium undergo cytological changes while in the ovules, the tapetal cells do not exhibit any cytological changes in their cell contents. This difference is due to the difference in the part played by them in supplying nutrition to the developing gametophytes. In the microsporangium, the tapetal cells themselves supply nutrition to the developing microspore mother-cells. The tapetum in the ovule, on the other hand, absorbs nutrition from the cells of the integument and passes it on to the endosperm, where it is stored up to be utilized by the developing embryo. Another difference between the tapetum in the anther sac and that in the ovule is that while in the former, the supply of nutrition is required only for a short period of time, in the latter, the supply of nutrition by the tapetum continues for a very long period, till the formation of the mature embryo. When the tapetum has completely absorbed and transmitted all the nutrition contained in the integumentary tissue, and there is no more of the integumentary tissue left, the tapetum in the ovule often takes on a protective role. A well-developed tapetum of integumentary origin, surrounding the embryo-sac, is present in most of the members of the Scrophulariaceæ, as also in many other plants of the Sympetalæ, mentioned earlier. The function of the tapetum, in most cases, is one of absorbing nutrition from the tissue of the integument, adjacent to it and transferring them to the endosperm tissue. Thus the tapetum has two distinct functions, firstly absorption of food materials from the integument and secondly their storage and transportation to the endosperm tissue. Besides these two functions, a third function has often been ascribed to it. In *Celsia* (Krishna Iyengar, 1939) and *Lobelia* (Kausik, 1938) the tapetum has been found to serve as a protective layer. As the epidermis of the testa in the mature seed is composed of thin-walled cells it serves no protective function. As a result, the task of developing a protective layer has fallen upon the tapetal tissue. The inner walls of the tapetal cells become thickened greatly and they seem to perform a protective function.

#### VIII. SUMMARY

1. The order of development of the floral parts in *Angelonia* and *Stemodia* is found to be calyx, stamens, corolla and pistil.
2. The ovary in *Angelonia*, *Stemodia* and *Vandellia* is bi-carpellary with axile placentæ, while in *Dopatrium*, it is uni-ocular and bi-carpellary, with parietal placentæ.

3. In all the members described above, a hypodermal archesporium directly functions as the megaspore mother-cell. A linear tetrad of megaspores is formed, the chalazal one being the functional one.

4. The mature eight-nucleate embryo-sac is essentially similar in all the above members, except in *Vandellia*, where the chalazal half of the embryo-sac is bent at an angle to the micropylar part and the micropylar part of the embryo-sac is very much elongated and swollen so as to form an extra-ovular embryo-sac.

5. A single layer of nucellar tissue which degenerates very soon is a characteristic feature of all the members described.

6. An integumentary tapetum is formed in all the members except in *Angelonia*, where it is absent. The tapetum has a nutritive function in all cases. The tapetum of the anther and the ovule are compared.

7. The antipodals are ephemeral in *Angelonia*, *Stemodia* and *Dopatrium*, while in *Vandellia* they are persistent. As a rule, the synergids degenerate after fertilization. But in *Angelonia*, they not only persist but also present an enlarged size, upto a late stage in embryo-formation. The possible correlation of this with the absence of haustoria of any kind, micropylar or chalazal, in the genus, is suggested. The haustoria are, however, the rule in the other genera investigated.

8. Details of endosperm development are given in the case of *Dopatrium*, *Stemodia* and *Vandellia*. The micropylar haustorium in all the members is four-nucleate, while the chalazal haustorium is composed of two uninucleate haustorial cells, which often fuse in *Vandellia*.

#### IX. ACKNOWLEDGEMENT

The work was carried out in the Botanical Laboratory of the Annamalai University. It is a source of sincere pleasure to record my grateful thanks to Dr. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S., F.R.M.S., Head of the Department of Botany, under whose guidance the investigations were made. I am grateful to the University authorities for the award of a studentship.

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## NATURE OF THE RESERVE FOOD IN SEEDS AND THEIR RESISTANCE TO HIGH TEMPERATURE\*

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Received for publication on August 24, 1940

### INTRODUCTION

MANY attempts have been made by various workers to find out the effect of heat on the viability of seeds. Some of them have applied heat to seeds previously soaked in water, while others have found out the effect of soil temperature on germination of various seeds. Livingston and Haasis refer to the effect of temperature on dry seeds thus:—"Test with seed samples that had not received any preliminary soaking at all gave results that were essentially like those secured with soaked seeds, excepting that the time period needed to give any specific germination percentage with a given maintained temperature was somewhat longer than unsoaked seeds were used as might be expected."

These and other authors were after finding out the germinating percentage with reference to temperature applied during the period of germination, but in the present work an attempt was made to find out the relation of reserve food and the resistance of seeds to high temperature as far as their capacity for germination was concerned. The seeds or the grains were incubated dry and were sown after incubation. A grain or seed was considered to have germinated when the white coleoptile or the radicle had emerged.

### MATERIALS AND METHODS

Starchy and oily seeds were experimented on. For oily seeds were taken 2 varieties of mustard (*Brassica campestris* L. Var *Sarson* and *B. Napus* Var *dichotoma*); cotton (*Gossypium herbaceum* L.); linseed (*Linum usitatissimum* L.); sesamum (*Sesamum indicum* etc.); and the sun-flower (*Helianthus annuus*); while for starchy seeds *Eleusine coracana* Gaertn; the Wheat (*Triticum vulgare* Vill.);

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\* Read before the Indian Science Congress at Indore in 1936.

the gram (*Cicer arietinum* L.); *Cucurbita Pepo* L. and *Lagenaria vulgaris* Ser. were used.

They were first of all tested for their germinating capacity. The samples which germinated well were chosen for experiments. Some dry seeds of each type were incubated in the electric oven (Chas. Hearson & Co., Ltd.) at a definite temperature for a certain length of time. 25 of each of these incubated seeds were sown separately with 25 unincubated seeds sown as control. The average of four such sets was recorded. Fluctuations of temperature during the experiments was not great and the record kept for the variation of day and night temperatures did not show much difference. So the experiments were under fairly identical environments.

The first experiment with 2 varieties of mustard and *Eleusine* showed that the mustard stands the higher temperature better than *Eleusine* (Table 1). Both varieties contain more than 45% of oils and *Eleusine coracana* is almost wholly a starchy seed, containing only 2% of oils. It is clear therefore that oily seeds resist higher temperature than starchy ones. Among themselves *B. napus* has higher resisting power than *B. campestris*.

TABLE 1

Showing germination at various temperatures in relation to percentage of oil content and thickness of the testa

Name of seed	Percentage of oil	Thickness of the testa or pericarp in $\mu$	Percentage of germination in experimental seeds					Percentage of germination in control
			40 C.	45 C.	50 C.	55 C.	60 C.	
<i>Eleusine coracana</i>	2.0	30.0	58	42	20	..	7.5	100
<i>Brassica sarson</i>	49.0	39.7	97.0	77	59	..	52.5	100
<i>Brassica napus</i>	48.2	53.1	100	90	90	..	80	100
<i>Triticum</i>	1.2	65.0 (pericarp)	95	90	70	50	15	80
<i>Sesamum</i>	45.0	79.6	100	100	90	90	80	90
<i>Linum</i>	25.0	88.5	98	96	80	90	80	90
<i>Cucurbita Pepo</i>	7.3	88.5	..	80	70	..	60	90
<i>Gossypium</i>	30.0	143.7	92	90	80	60	60	100
<i>Lagenaria vulgaris</i>	1.5	619.0	..	95	85	..	90	90

To find the cause why *B. napus* resists higher temperature better than *B. campestris*, the percentage of fats and oils were estimated before and after incubation. *B. napus* contained 48.2% of oils and *B. campestris* 49.01% before incubation. The percentage after incubating the seeds at 60° C. was 47.01 in both the cases. It was thus clear that the difference in oil content is not the cause here.

Sections were cut of the testa of the two varieties. *B. napus* had a testa of 53.1 $\mu$  in thickness while *B. campestris* had one of 39.7 $\mu$ . This indicates that the thickness of the testa might account for the better resistance of *B. napus*. This conclusion was confirmed by later experiments.

For the next experiment the wheat and the gram were chosen for the starchy seeds and cotton for oily ones. The incubation period, temperature and germination percentage are noted in Table 1. From these readings it is clear that the cotton seeds with fatty reserve resist the deleterious effect of high temperature better than the starchy seeds—the wheat and the gram. Among the starchy seeds themselves, namely wheat and gram, the wheat can endure higher temperature better than the gram—because the pericarp of the former is thicker (65 $\mu$ ) than the testa of the latter (37.5 $\mu$ ) and perhaps also because the wheat contains 1.2% of oil while the gram contains none. Fig. 1 shows the relation of the percentage of oil to the power of resistance to high temperature.

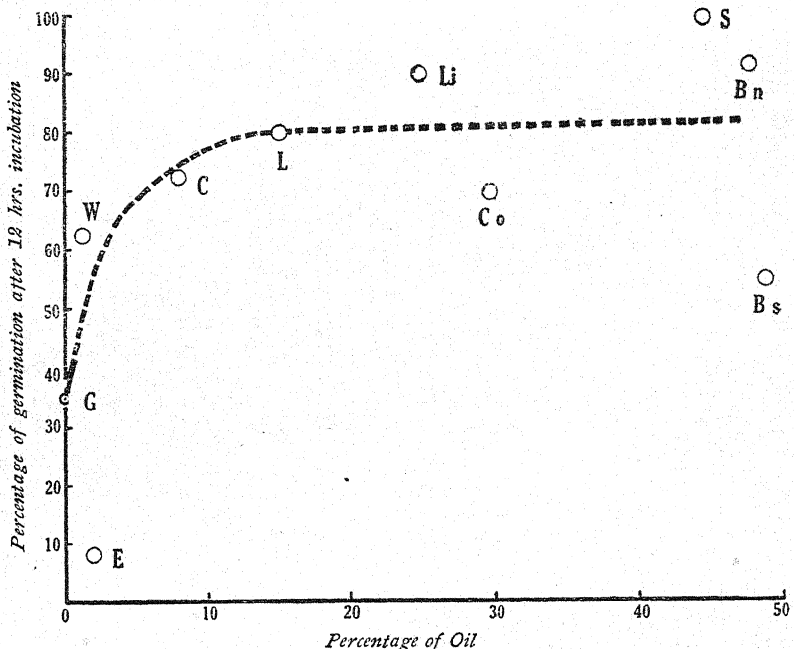


Fig. 1. Showing the relation of percentage of oil in the seed and percentage of germination after the seeds have been kept at 60° C. for 12 hours: The broken line indicates the probable relation. E = *Eleusine*, G = *Gram*, W = *Wheat*, C = *Cucurbita*, L = *Lagenaria*, Li = *Linum*, Co = *Cotton*, S = *Sesamum*, Bn = *Brassica napus*, Bs = *Brassica sarson*.



To have a definite idea of the part played by the thickness of the testa, experiments were made with *Cucurbita* and *Lagenaria* seeds both starchy and containing less than 20% of oil. From the table it will be seen that *Cucurbita* had a testa  $88.5\mu$  thick while *Lagenaria* had  $619.5\mu$ . The greater resistance to high temperature resulting in greater percentage of germination in the case of *Lagenaria* could be ascribed to its thicker testa. The experiment was repeated with the same result. Oily seeds like *B. campestris* and *Sesamum indicum* were next experimented. They had nearly the same percentage of oils, namely 48.2% and 45% respectively. Figures in Table 1 show that as the thickness of the testa of *Sesamum* is greater, the viability with reference to high temperature is also greater. Fig. 2 shows the relation of the thickness of the testa to the power of resistance to high temperature.

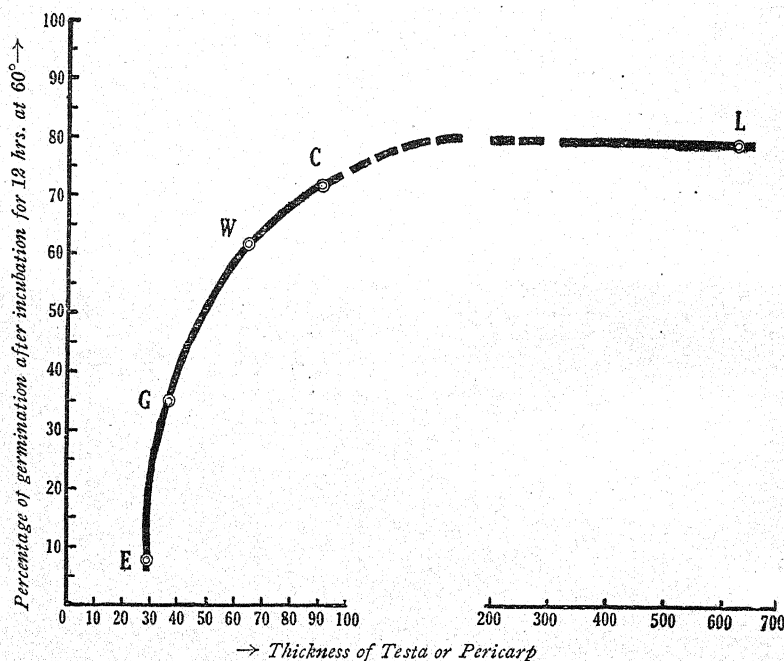


Fig. 2. Showing the relation between the thickness of the testa or pericarp and the percentage of germination after the seeds have been kept at  $60^{\circ}\text{C}$ . for 12 hours: The broken portion of the curve indicates the probable course of the curves. E = Eleusine, G = Gram, W = Wheat, C = *Cucurbita* and L = *Lagenaria*.

In order to see if thicker testa checks loss of water during incubation, loss of water was measured after incubation and the results are shown in Fig. 3. It is clear that seeds with thicker testa lose less water than those with thinner ones.

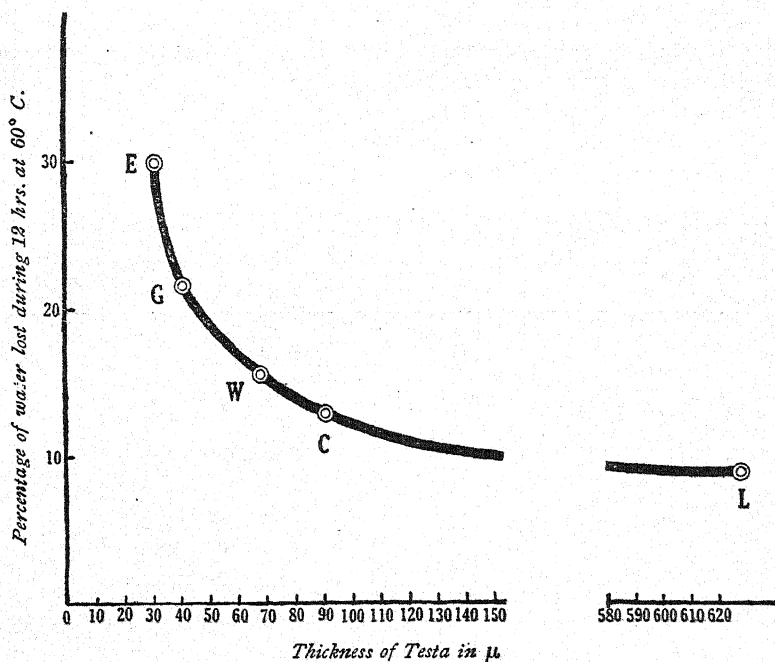


Fig. 3. Showing the relation of the thickness of the testa in  $\mu$  and the percentage of water lost during 12 hours at  $60^{\circ}\text{C}$ : E = *Eleusine*, G = *Gram*, W = *Wheat*, C = *Cucurbita*, L = *Lagenaria*.

The nature of the reserve food also seems to have some relation to loss of water. Starchy seeds with the same thickness of testa lose more water than oily seeds.

The relation of the period of incubation on viability and also the relation of the percentage of oil and viability were next examined. Seeds with different percentages of oil were incubated for the same period and also the same oil seeds were incubated for different periods.

It was noted that the figures for *Sesamum* are always higher than those for linseeds. It was also seen that the germination percentages fell off in both the cases as the incubation periods became longer.

Cotton seeds and linseeds were next compared.

It was found that although cotton contains more oil than the linseed, the viability of the latter is greater than that of the former. The only explanation that suggests itself is that the thin mucilage coating on the linseed enables it to endure the high temperature without losing its viability. When the thickness of the testa of linseed was measured together with the mucilage coating it was found to be  $117.7\mu$  and the mucilage coating alone was nearly  $30\mu$ .

In order to establish the relation of the period of incubation and viability a series of experiments were performed with two varieties of sun flower seeds.—

They were kept for incubation at 60° C. and 40° C. separately in 2 incubators.

The percentage of germination are shown in Table 2.

TABLE 2  
*Showing the Relation between Period of Incubation  
of Dry Seeds and their Viability*

Description of seed	Temperature	Incubation period in hours						Control
		8	12	24	48	72	120	
Sun flower (large)	60° C.	60	60	30	12	4	0	
	40° C.	80	92	60	36	30	30	
Sun flower (small)	60° C.	80	48	50	24	0	0	
	40° C.	96	100	80	32	24	10	

These figures show that viability of seeds decreases after continued incubation and is at last lost altogether.

Another feature is, as is to be expected, that incubation for a shorter period at a higher temperature has the same effect as incubation for a longer period at a lower temperature.

#### SUMMARY

The effect of temperature on the viability of starchy and oily seeds has been studied. The seeds were exposed to 40° C., 45° C., 50° C. and 60° C. for periods ranging from 8 hours to 120 hours. It has been found that :

(1) Oily seeds resist the effect of high temperature better than starchy ones.

(2) Among the oily seeds the greater the oil content, the higher the temperature they resist.

(3) Viability also depends on the thickness of the testa ; the thicker the testa is, the more viable is the seed in relation to temperature. This holds good in case of all seeds, both oily and starchy ones. Thick testa checks loss of water during incubation.

(4) Mucilage coating on testa affects the percentage of germination after incubation and

(5) Continued incubation decreases viability quickly at higher temperature and slowly at lower ones.

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## PERMEABILITY OF THE WALL OF THE XYLEM VESSEL\*

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Received for publication on September 4, 1940

### INTRODUCTION

It is well known from very early days that roots absorb water and nutrients from the soil. But the path of the transport of the solutes from the roots to the leaves has been under dispute. According to one view, the movement of the nutrients occurs mainly through xylem along with the transpiration stream. The other view is that it occurs mainly through the phloem. Considerable experimental work has been done, mainly by ringing different parts of the shoot, to substantiate the one or the other of the two views. Mason and Phyllis<sup>3</sup> after a thorough review of the problem of translocation conclude that "ringing experiments have shown that soil solutes ascend up the stem in the wood; but they have not demonstrated that they may not also ascend in the phloem. It must be admitted, however, that the evidence available at hand renders it unlikely that they normally do so."

Even those who accept the view that translocation is normally effected through the phloem, think that under special conditions, the rate of transpiration influences both absorption and transport of solutes. Thus they think that if the solutes are in excess in the soil, living cells absorb the maximum quantity and after their accumulation capacity is satisfied, the excess passes into the transpiration stream.

It is thus generally assumed that the living cells adjoining the path of the transpiration stream can get a supply of the solutes by extracting them from the transpiration stream. The observations made by Ekambaram and Rao<sup>2</sup> on the effects of Formalin,  $\text{KNO}_3$  and  $\text{NaCl}$  on cut shoots of *Barleria cristata* threw doubts on this general assumption. It was therefore decided to study the problem of solute movement through the vessel walls in a more detailed manner. This paper gives the results of a study on the problem of solute movement through the xylem vessel walls.

### METHODS AND MATERIALS

Shoots of *Tecoma stans* were cut under water at about 7 A.M., and brought into the laboratory and fresh cuts were made inside

\* Contribution from the Presidency College Botany Laboratory, Madras.

the laboratory under water. These were then allowed to remain with their cut ends in water for another two hours. At about 9 A.M. they were transferred to 10 per cent. solutions of different chemicals and kept under observation. The method adopted was based on observations previously made, that as the solution reached the living cells, the solutes tended to accumulate in them, eventually causing death of the cells, which was indicated by their discolouration. Ordinarily the beginning and the progress of discolouration could easily be followed, especially in the leaves. Discolouration appeared from about half an hour to two hours depending on the solute. In all, six different acids and eighteen salts were used.

### RESULTS

According to the region where death of the cells occurred first, the different chemicals used fall under two main types of killing :

1. The Acetic acid type and
2. The Potassium nitrate type.

#### THE ACETIC ACID TYPE

As the acid passed up, the stem and the lower petioles became discoloured. In the lamina the discolouration spread along the main veins and soon followed the reticulate pattern of the lateral veins and the veinlets. In about an hour, one could see the reticulate arrangement of the discoloured areas in the lamina (Pl. VII, Fig. 1).

#### THE POTASSIUM NITRATE TYPE

In this type the first appearance of discolouration was not in the stem or petioles as in Type I described above, but in the cells of the lamina. The first indication was that the mesophyll cells in between the lateral veins turned darker than the rest. Soon small groups of cells were seen to get discoloured in different parts of the lamina. One noticeable feature at this stage was that there was always a vein ending in each discoloured patch. As more solution was absorbed, the discolouration became more and more prominent and all the mesophyll tissue except strips of tissue along the veins turned brown. The green colour of these narrow strips along the veins was in striking contrast with the brown mesophyll tissue (Pl. VII, Fig. 2).

The different chemicals used and falling under the two types are given below :—

##### *Acetic acid type*

1. Acetic acid
2. Formalin
3. Picric acid
4. Sulphuric acid
5. Pyrogalic acid
6. Boric acid
7. Mercuric chloride

##### *Potassium nitrate type*

1. Potassium nitrate
2. Calcium nitrate
3. Sodium phosphate
4. Potassium phosphate
5. Potassium acid phosphate
6. Potassium bromide
7. Potassium bromate

*Acetic acid type*

8. Sodium carbonate
9. Potassium carbonate
10. Sodium bisulphate
11. Ammonium nitrate
12. Silver nitrate

*Potassium nitrate type*

8. Potassium chlorate
9. Potassium iodide
10. Magnesium sulphate
11. Sodium acetate
12. Sodium nitrate

The appearance of the shoot as a whole after treatment with the different chemicals showed certain differences. In the Acetic acid type, there was no wilting of the leaves but the younger nodes and the bases of the petioles became flaccid and the leaves as a whole drooped down. In the second type, the first visible effect was a wilting and drooping of the leaflets at the younger parts of the shoots. With boric acid, there was a curling of the leaflets long before the discolouration along the veins appeared. With magnesium sulphate, there was neither curling nor wilting of the leaflets at any stage.

## DISCUSSION

Caroline Rumbold<sup>4</sup> has recorded different types of killing of the leaf tissues in the chestnut, when the trees were injected with different kinds of chemicals. Her observations are of a general nature and they do not throw light on the problem of the permeability of vessel walls.

In the present study the chemicals were supplied to the shoots through the cut end along with the transpiration stream. If, as in the Acetic acid type, the walls of the vessels and of the adjoining living cells were permeable to the solute, then the solute diffused out into the living cells. This naturally resulted in the rapid accumulation of the solute in the living cells adjoining the vessels and finally in their death. In the Potassium nitrate type, the discolouration and death appeared first in the mesophyll cells situated at the ends of the veinlets and not along the veins. It might therefore be concluded that the two types of killing were due to the differences in the permeability of the xylem vessel walls to the different kinds of solutes experimented with. Where the vessel wall was permeable to the solute, the cells adjoining the vessels were killed first and where it was not permeable, the cells at the vein endings were the first to suffer :

Thus the important point that arises out of the present study is that the xylem vessel walls are not equally permeable to all the solutes that may be carried along the transpiration stream in a plant. From the lists of chemicals given under the two types, it is not possible at present to make out whether there is any relation between the nature of the chemical and its diffusibility through the vessel walls. But it is evident that only certain solutes can pass out of or into the vessels. The characteristics of the xylem vessel wall has not been realised by the various investigators so far. Before one assumes the movement of solutes from the xylem to the living cells or from the living cells into the vessels, the permeability of the vessel walls to the particular solutes has to be determined.



## SUMMARY

1. Cut ends of the shoots of *Tecoma stans* were placed in 10 per cent. solutions of twenty-four different chemicals.

2. As the solutions ascended into the shoot, two types of killing of the living cells in the stem and leaves were observed.

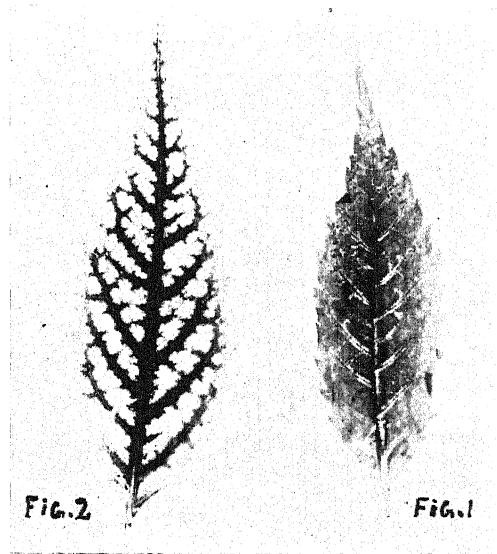
(a) *The Acetic Acid type* in which the cells adjoining the xylem vessels in the stem and the cells along the veins in the leaves were first killed, and

(b) *The Potassium Nitrate type*, in which the cells in the stem along the vessels were not killed first, but the killing started in the groups of mesophyll cells at the vein endings.

3. It is therefore inferred that the wall of the xylem vessel is not equally permeable to all the different solutes present in the transpiration stream, as is generally assumed.

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DR. T. EKAMBARAM  
AND  
MISS V. K. KAMALAM

*PERMEABILITY OF THE WALL OF THE XYLEM VESSEL*



## NOTE TO CONTRIBUTORS

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Only papers written or *communicated by* Members of the Indian Botanical Society are published in the Journal.

Attention to the following points will greatly assist the Editor and ensure early and satisfactory publication :—

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In view of the high cost of publication, contributions should be as concise as possible and all unnecessary tables and illustrations should be avoided. If the contributions are very long, the authors may be required to contribute a portion of the cost of publication.

Names of genera and species should be underlined and will appear in italics. The names of the authors of genera and species should always be given.

Original papers must conclude with a summary, drawing attention to the main facts and conclusions. References to literature cited should, as far as possible, be complete and must be carefully verified. A bibliography should be given at the end of the paper arranged alphabetically under authors' names.

References to literature in the text should be made by quoting the author's name and the year of publication adding the page where possible, thus (A. B. 1934, p. 25). When the author's name occurs as a part of the text, only the year and page need be given. No references should be given as footnotes.

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# The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

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VOL. XIX]

DECEMBER, 1940

[Nos. 5 & 6

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## THE ROOT-STRUCTURE OF *CHLOROPHYTUM TUBEROSUM* BAKER

BY D. P. MULLAN

*St. Xavier's College, Bombay*

Received for publication on September 21, 1940

*Chlorophytum tuberosum* Baker (*Phalangium tuberosum* Kunth.), a member of the Liliaceæ, is found growing on gravelly or sandy soil, or on open forest lands. The plant is a herb with a small root-stock bearing a number of short, fascicled roots which are confined to the comparatively dry surface layers of the soil. The root-fibres are fleshy. The majority of roots swell locally, at the distal end, and form ellipsoidal tubers (Fig. 1). At the break of the monsoon the plant puts forth a cluster of broadly-linear, radical leaves with undulating margins. Bunches of pure white flowers are borne on a naked scape during the rainy season—about the month of August in the Bombay Presidency.

During the dry season the plant dies down to the level of the soil. The aerial parts disappear but the root-stock and the tuberous roots remain underground. At the expense of the reserve material of some of the tuberous roots, the plant produces new leaves and flowers in the beginning of the next rainy season. Thus the roots of *C. tuberosum* are not annual organs, as is the case with other monocotyledons, but act as perennating organs, enabling the plant to tide over the dry season. The anatomy of the root is described in this paper.

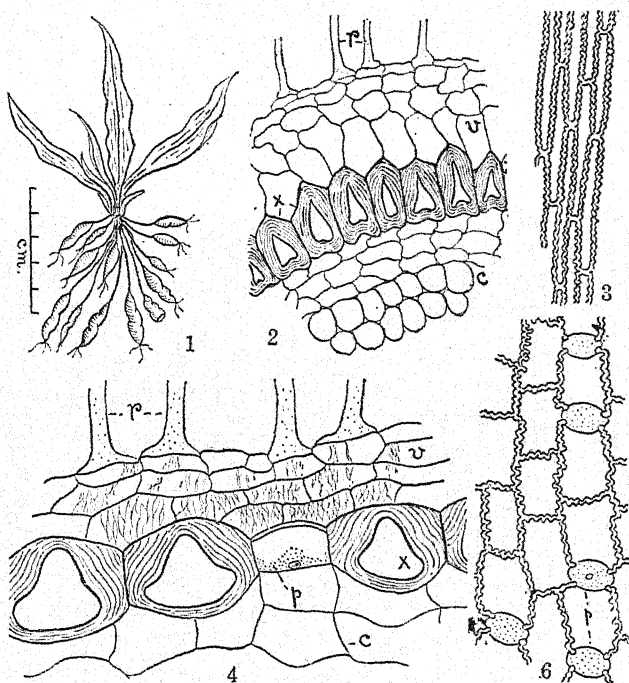
The roots consist of two parts: the cylindrical proximal part and the distal tuberous part. A peculiarity of the roots is that the root-hairs are not confined to a definite area, as is the case in many roots, but are found all over the root, covering both the slender as well as the tuberous parts. Haberlandt (1914, p. 219) is of opinion that plants living in a low degree of humidity are characterized by the constant presence of persistent root-hairs. Eames (1925, p. 229) believes that "the presence of persistent root-hairs is correlated with

the comparative absence of secondary growth and the lack of periderm formation".

The slender proximal part of the root is covered by a multiple piliferous layer consisting of four layers of cells, the outermost bearing the root-hairs. The cells of the two inner layers are radially stretched, while the outer cells become irregular due to lateral pressure (Fig. 2). The cells are devoid of contents and are in uninterrupted contact with one another. From its fuller development in the region of the tuberous part, the multiple piliferous layer may be regarded as of the nature of the velamen tissue found in the aerial roots of many epiphytic Orchids and some epiphytic Aroids. The velamen tissue in the proximal part of the root differs from that of the epiphytic Orchids by mostly lacking in the characteristic reticulate thickening fibres of the cell walls. The multiple piliferous layer is separated from the cortex by a well developed exodermis. The latter is composed of a single layer of prominent cells with narrow lumina and strongly thickened, lignified walls (Fig. 2). In a cross-section the cells appear more or less squarish in outline, while in surface view, *i.e.*, in tangential longitudinal section, they are seen to be vertically elongated and the side walls are folded in a sinuous manner (Fig. 3). In the proximal part of the root the exodermis is composed of uniform sclerosed cells, there being no passage-cells. The cortex is composed of about twenty layers of clear, rounded, thin-walled cells. The inner cortex is characterized by the development of radially placed lacunae. The endodermis is thin-walled throughout. The outermost 3-4 layers of the cortex, *i.e.*, the layers immediately under the exodermis, consist of cells with thin but lignified walls, while the rest of the cortical cells have walls of cellulose. A single layer of cells separates the protoxylem groups from the endodermis and constitutes the pericycle. The stele is 11- to 14-arch. The conjunctive tissue around the vascular bundles shows the presence of sclerosed fibres. The pith consists of thin-walled cells.

A comparison of the cross-section of the slender proximal part with that of the tuberous part of the root shows various modifications. The tubers are seen to owe their swelling mainly to the expansion of the cortical parenchyma, the cells of which elongate in a radial direction (Fig. 7), forming a massive aqueous tissue. The cortical cells are clear and show the presence of prominent nuclei. Due to the expansion of the cells, the cortical lacunae around the stele are mostly obliterated. The cortex is traversed by crystal-sacs, holding bundles of raphides. The outermost 3-4 layers of the cortex are tangentially compressed and show thin, lignified walls as in the proximal part of the root. In a few tubers the inner cortex is seen to be strengthened by stone-cells, occurring singly or in groups. This is not, however, a constant feature of most tubers. In the tuberous part of the root, the exodermis is more fully developed, being composed of large cells with wide lumina. Towards the base of the tuber, *i.e.*, where the latter joins the slender proximal part of the root, the walls of the exodermis are

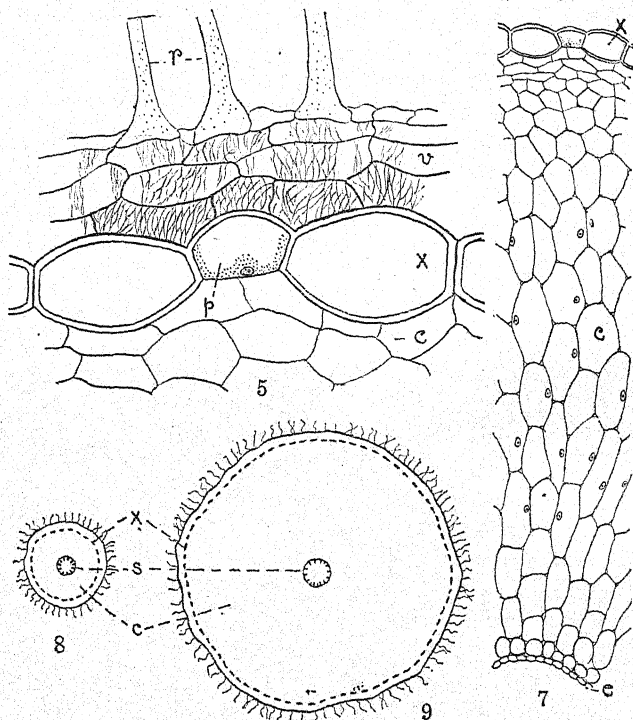
strongly thickened (Fig. 4) while in the major part of the tuber the cell-walls are thin (Fig. 5) as compared with those of the proximal part of the root. In surface view, *i.e.*, in tangential longitudinal section, the cells of the exodermis do not appear to be vertically elongated though the lateral walls are sinuous as in the proximal part of the root (Fig. 6). A characteristic feature of the exodermis of the tuberous part is the presence of passage-cells. The latter occur in vertical series, being interspersed among the larger exodermal cells (Fig. 6). The passage-cells show prominent nuclei and have thin inner and side walls (Figs. 4, 5). Figs. 2 and 5, or Figs. 3 and 6, magnified equally, indicate the contrast in size and structure of the exodermis in the proximal and tuberous parts of the same root. The multiple piliferous layer outside the exodermis is also more fully developed in the tuberous part of the root and consists of four layers of empty, tangentially-stretched cells in uninterrupted contact, the outermost bearing the root-hairs. Especially towards the apical region of the tuber, the multiple piliferous layer is more



Figs. 1-4 and 6. *Chlorophytum tuberosum* Baker.—Fig. 1. The plant growing in sandy soil. Fig. 2. T. S. proximal part of the root. *r*, root-hair; *v*, multiple piliferous layer; *x*, exodermis; *c*, cortex ( $\times 240$ ). Fig. 3. Tangential L. S. proximal part of the root, showing the exodermis in surface view ( $\times 82$ ). Fig. 4. T. S. through basal part of tuber. *r*, root-hair; *v*, velamen; *x*, exodermis; *p*, passage-cell; *c*, cortex ( $\times 240$ ). Fig. 6. Tangential L. S. tuber, showing exodermis in surface view. *p*, passage-cells ( $\times 82$ ).



typically developed in that the cell-walls exhibit the characteristic delicate reticulate thickening peculiar to the velamen tissue of the epiphytic Orchids (Fig. 5). In the tuberous part, the stele also expands a little. A comparison of Figs. 5 and 6, magnified equally, brings out the contrast in size of the cortex and the stele of the slender proximal part and of the dilated tuberous part of the same root. The increase in the size of the stele is brought about mainly by the expansion of the thin-walled cells of the pith. The stele is 12- to 15-arch, one or two extra protoxylem groups appearing at times in the dilated part of the root. The sclerosed fibres of the conjunctive tissue of the proximal part are absent in the tuber. Lateral roots develop towards the apex of the tuber at the beginning of the rains. Such roots have their origin in the pericycle.



Figs. 5 and 7-9. *Chlorophytum tuberosum* Baker.—Fig. 5. T. S. tuber. r, root-hair; v, velamen; x, exodermis; p, passage-cell; c, cortex ( $\times 240$ ). Fig. 7. T. S. tuber. e, endodermis; c, cortex; x, exodermis ( $\times 82$ ). Fig. 8. T. S. proximal part of the root, showing the relation of stele (s) to cortex (c). x, exodermis ( $\times 13$ ). Fig. 9. T. S. tuberous part of the root, showing the relation of stele (s) to cortex (c). x, exodermis ( $\times 13$ ).

The proximal part of the root is adapted mainly for the function of anchorage as is seen from the greater degree of sclerosis in the more strongly thickened and lignified exodermis and conjunctive tissue. The major portion of the tuberous part of the root is

constructed for storage and is adapted for rapid absorption and conduction of water as is evidenced by a typically developed velamen, by the presence of passage-cells, and by the less thickened and large-celled exodermis. The latter is easily pierced by the newly-developed roots near the apex of the tuber.

A remarkable feature of the root-structure of *C. tuberosum* is the presence of velamen in its terrestrial roots. Schimper (1903), Goebel (1905), Haberlandt (1914) and others have described the velamen tissue as a special apparatus for the absorption of water from the air by the aerial roots of tropical epiphytic Orchids and of certain epiphytic Aroids. The occurrence of a similar velamen tissue in the subterranean roots is shown by Holm (1904, pp. 203, 204), in the cases of North American terrestrial Orchids like *Tipularia discolor* Nutt. and *Bletia verecunda* Sw. Moss (1923) has also given an account of "the presence of velaminous roots in the terrestrial Orchids, especially noticeable in the Orchid genus *Eulophia*, abundant at the Cape". Among plants other than the terrestrial Orchids, Arber (1925) has shown the occurrence of velamen in the terrestrial roots of *Crinum Powellii* Hort., *Asparagus Sprengeri* Reg., *Aspidistra elatior* Blume and *Semele androgyna* (L.) Kunth. That the multiple piliferous layer of the terrestrial roots of *C. tuberosum* is of the nature of a velamen tissue is seen not only from its structure but also from its ability to absorb water quickly. If the tuberous roots are dried and immersed in a coloured solution, it is seen that the coloured liquid penetrates rapidly the velamen tissue and is later absorbed by the passage-cells of the exodermis.

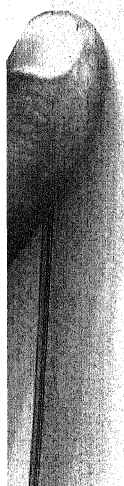
#### SUMMARY

The anatomy of the tuberous roots of *Chlorophytum tuberosum* Baker is described.

The terrestrial roots of the plant show the presence of a velamen and an exodermis similar to those of the aerial roots of some epiphytic Orchids and Aroids.

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**CLADOSPONGIA, A NEW MEMBER OF THE  
CRASPEDOMONADACEAE FROM MADRAS\***

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Received for publication on August 15, 1940

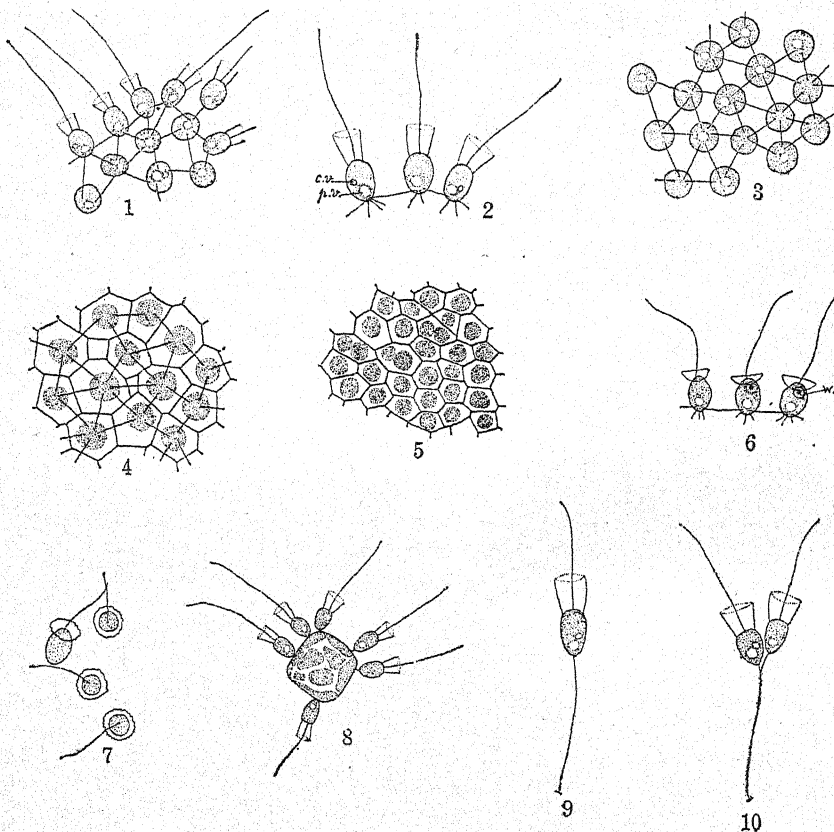
In some water collected from a small pool near the Cooum estuary at Madras and kept in the laboratory for a few days, some small tufted, whitish growths were found attached to the sides and bottom of the glass vessels. These whitish outgrowths on examination proved to be a colonial colourless flagellate evidently belonging to the family Craspedomonadaceae (Pl. VIII, Fig. 1). The colony is cylindrical and branched and the branches become gradually narrowed towards the apex (Pl. VIII, Figs. 1, 2). The whole colony is attached at the bottom by means of a colourless gelatinous substance (Pl. VIII, Fig. 1).

The colony consists of a large number of unicellular colourless cells each provided at the anterior end with a hyaline, collar-like outgrowth of the protoplast and a single long flagellum arising from its apex (Text-figs. 1, 2, 6, 7; Pl. VIII, Fig. 5). The individual cells are arranged close to one another around the periphery of the cylindrical branches, with their ciliary ends pointing outwards (Pl. VIII, Figs. 2, 5). The central core of these branches consists of a colourless mucilaginous substance. A careful examination of the peripheral region of these branches shows that these organisms are not lying merely embedded in the general mucilaginous ground substance, but are united with one another by means of delicate protoplasmic connections not quite unlike those seen in several species of *Volvox* (Pl. VIII, Figs. 3, 5, 6; Text-figs. 1-4, 6). These protoplasmic connections are seen both in the living material and also in material properly killed and stained (Text-figs. 1-4, 6). These connections arise, not from the sides of the cells as in *Volvox*, but are seen starting a little towards the posterior end of the cells (Pl. VIII, Figs. 3, 5, 6; Text-figs. 1-4, 6). As many as 4-7 such protoplasmic connections arise from each cell (Pl. VIII, Figs. 5, 6; Text-figs. 1-4, 6). A tiny knot could be distinguished in the middle of these connecting strands somewhat similar to what is seen in the intercellular connections of *Volvox*. In material stained with a dilute solution of methylene blue, gentian violet or Loeffler's blue, there is seen in addition to these protoplasmic connections a number of polygonal mucilaginous envelopes round each protoplast similar to what

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\* From the University Botany Laboratory, Madras.

one sees in *Volvox* (Iyengar, 1933 ; Pocock, 1933) when it is similarly stained (Pl. VIII, Fig. 4 ; Text-figs. 4-5). These envelopes are really the cell membranes which are mucilaginous and which through mutual pressure appear polygonal. The delicate protoplasmic strands connecting the adjoining cells pass through the thick mucilaginous walls (Text-fig. 4) and the strand appears slightly thickened at the place of meeting of the mucilaginous walls of the two neighbouring cells.



Text-figs. 1-10. *Cladospongia elegans* gen. et sp. nov.—Fig. 1. Portion of a colony, showing the individual cells and their intercellular protoplasmic connections. Fig. 2. A few cells showing the protoplasmic connections in side view: note the vacuoles. Fig. 3. Cells showing intercellular protoplasmic connections from the posterior portion of the cells (taken at a lower focus). Fig. 4. Portion of the colony after treatment with iodine and staining with gentian violet, showing the intercellular protoplasmic connections and the thick mucilaginous cell walls. Fig. 5. Portion of the colony after staining with methylene blue, showing the mucilaginous cell partitions: some of the protoplasts have already divided into two. Fig. 6. A few cells of the colony after staining with gentian violet, showing the collar, the intercellular protoplasmic connections and

The individual cells of the colony are rounded in vertical view and rounded to ellipsoid in side view and are  $6.6-8.4\ \mu$  broad and  $8.4-10.1\ \mu$  long. The collar is nearly as long as the length of the body ( $6.6-8.4\ \mu$ ), while its diameter at the anterior end is about  $8.4\ \mu$ . The cilium is long and measures nearly 3-4 times the length of the body. Each cell has a single nucleus at its anterior end (Text-fig. 6) and two vacuoles towards its posterior end (Text-figs. 1-2). Of these two vacuoles one is larger than the other and does not appear to be contractile (Text-fig. 2, *p.v.*). The other is smaller and is found close to the bigger one and is actively contractile (Text-fig. 2, *c.v.*). Every time the smaller vacuole contracts, there appears to be a slight increase in the size of the larger permanent vacuole. It looks as though the contents of the smaller vacuole gets discharged into the larger one. Bütschli (1883-87, p. 889) in his account of the Choanoflagellata states that only one of the vacuoles is contractile, while the other is not and serves as a so-called "Schling-vacuole". Our observations in the present form agree quite well with those of Bütschli.

The fully developed colony measures about 2-4 mm. long and about 0.2-0.3 mm. at its broadest portion. The branches arise from any portion of the colony and are really lateral to begin with. But they soon grow rapidly and become directed forward and give a false dichotomous appearance (Pl. VIII, Figs. 1, 2). In the living material, the branches of the colony show a slight swaying movement, evidently due to the combined action of the cilia of the individual cells. This movement is especially well seen in some of the terminal branches of the colony.

Multiplication takes place by the division of the individual cells into two. The plane of division is evidently longitudinal, since the two daughter protoplasts are seen lying side by side, inside a common mucilaginous cell membrane (Pl. VIII, Fig. 4; Text-fig. 5). Occasionally the colony gets dissociated into individual cells, which are then seen swimming about freely in the water. These individuals after sometime come to rest on various objects in the water. They often came to rest on the individual cells of a species of *Cyclotella* which were common in the water. Often as many as seven to eight individuals were found attached to a single individual *Cyclotella* cell (Text-fig. 8). At first these individual cells were attached to the substratum by means of their posterior end, when they looked very much like individuals of *Monosiga*. But they soon developed short stalks from their posterior end which gradually became longer, until finally they were nearly 3-4 times as long as the body (Text-figs. 8, 9). In a few cases, the stalks bore two or three cells at their tips evidently through the division of the original cell. At this stage

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the nucleus. Fig. 7. A few cells of the colony after staining with gentian violet, showing the somewhat crenate margin of the collar, when seen from above. Fig. 8. Some of the individual cells of the colony settled down on a cell of *Cyclotella*. Figs. 9 and 10. Individual cells showing formation of stalked colonies after settling down. *c.v.*, contractile vacuole; *p.v.*, permanent vacuole; *n.*, nucleus. Figs. 5 and 8 ( $\times 485$ ); rest ( $\times 715$ ).

they showed a certain amount of resemblance to *Codonosiga* (Text-fig. 10). The further fate of these stalked individuals could not be followed, since the organism began to deteriorate and disappear at this stage.

#### DISCUSSION

The present form in the structure of its individual cells with the protoplast, collar and single cilium clearly shows that it belongs to the family Craspedomonadaceae among the colourless flagellata. The individual cells of the colony are somewhat similar to those of *Monosiga* or *Codonosiga*, but in the general structure and appearance of the colony, it is quite unlike any member of the Craspedomonadaceae so far known. In the general organisation of the colony, it has reached a very high level, far higher than that reached by any other member of the Protomastiginae. Lemmermann (1914) has given a brief summary of the several stages of advance in colonial organisation seen within the group, Protomastiginae (Lemmermann, 1914, p. 55). He has traced the advance from colonies of two cells or more, to large colonies consisting of numerous cells variously disposed. Taking even the most highly developed among them, *e.g.*, *Desmarella*, *Sphaeroeca*, *Protospongia*, *Phalansterium*, *Rhipidodendron*, etc., each one of which has attained a complex development in its own way, one does not find in any of them any intercellular protoplasmic connections as are seen in the present form. None of them has reached the high state of organisation as seen in the present organism. The present form resembles in its complexity of structure the green alga *Volvox* in several respects. Just as *Volvox* may be considered to be the highest development in colonial organisation among the Volvocales, this form may be considered to be the highest expression of colonial organisation so far known among the Protomastiginae. The form may therefore be described as a new genus by name *Cladospongia* and placed in the family Craspedomonadaceae close to *Protospongia* and *Sphaeroeca*.

#### DESCRIPTION

##### *Cladospongia* gen. nov.

Colony cylindrical, branched once or more; branches more or less dichotomous; branches broader towards the base and narrowed towards the tip; the apex of the branches broadly rounded to obtusely conical; colony attached to the substratum by mucilage; cells embedded in a general mucilaginous matrix and arranged around the periphery of the cylindrical colony, enclosing a central mucilaginous core; each cell possessing a single transparent collar at the anterior end and a flagellum arising from its apex; cells of the colony connected with one another by delicate protoplasmic strands. Reproduction not known.

##### *Cladospongia elegans* sp. nov.

General characters same as those of the genus; colony 2-4 mm. long; branches of the colony 210-280  $\mu$  broad at the base and 42-70  $\mu$



near the tip; cells of the colony rounded to ellipsoid in side view; cells  $6.6-8.4\ \mu$  broad and  $8.4-10.1\ \mu$  long; plasmatic collar nearly as long as the body; cilia 3-4 times the length of the cell; protoplasmic connections 4-7 in number and starting from near the posterior end of each cell; reproduction not known.

*Hab.*—Growing in clusters on the sides and bottom of a glass culture vessel containing some pond water, Madras.

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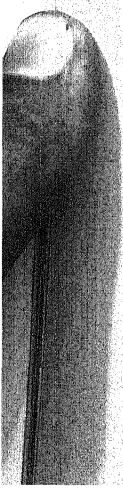
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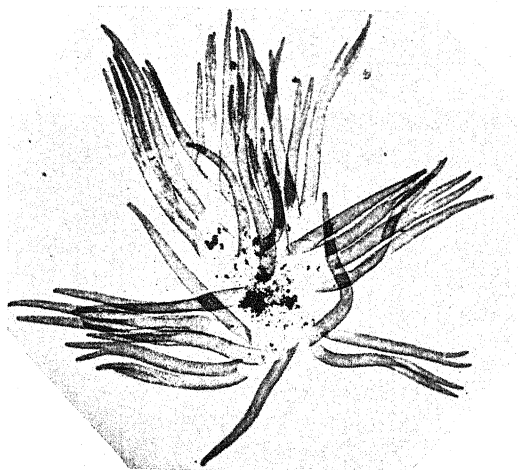
## EXPLANATION OF PLATE VIII

*Cladospongia elegans* gen. et sp. nov.

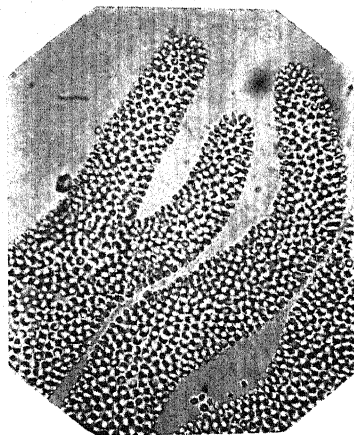
- FIG. 1. Photomicrograph of a living colony showing the dichotomous branches.  $\times 11$ .  
 FIG. 2. Photomicrograph of a few branches of the colony somewhat enlarged, showing the general disposition of the individual cells (Living material).  $\times 150$ .  
 FIG. 3. A few cells at the periphery of the colony, showing the protoplasmic connections starting from the lower side of the cells.  $\times 500$ .  
 FIG. 4. Portion of the colony showing the mucilaginous cell walls after staining with methylene blue. The protoplast has divided into two in some of the cells.  $\times 500$ .  
 FIG. 5. Marginal portion of a living colony showing the individual cells, with the collar, cilia and the protoplasmic connections from their posterior portions.  $\times 735$ .  
 FIG. 6. Portion of the colony showing protoplasmic connections between the cells.  $\times 800$ .



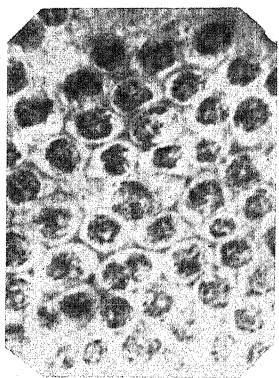




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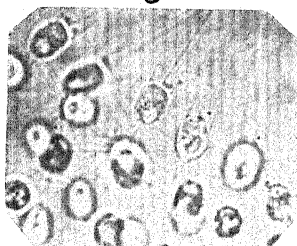
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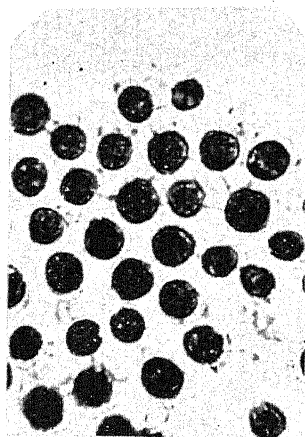
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M. O. P. IYENGAR  
AND  
K. R. RAMANATHAN *CLADOSPONGIA ELEGANS* GEN. ET SP. NOV.



## ZYGOGONIUM KUMAOENSIS, A NEW SPECIES OF ZYGOGONIUM FROM KUMAON

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Received for publication on September 4, 1940

THIS alga was found growing at the sides of rocks on red clay in the form of a whitish felt-like covering near Village Jariakhali, Almora, Kumaon hills, at an altitude of about 6,500 feet above sea-level on 20th September 1939.

In some plants the thallus is composed of a creeping prostrate portion and a more or less erect projecting portion (Fig. 1). The function of the prostrate system is that of fixation and support, and its cells give out rhizoids of different shapes. In some plants however there is no such differentiation into prostrate and projecting portions. In shallow soil the rhizoids are more or less knob-like (Figs. 3 and 5) and deeper down they are fairly long (Fig. 4). In rare cases rhizoids may even be two-celled.

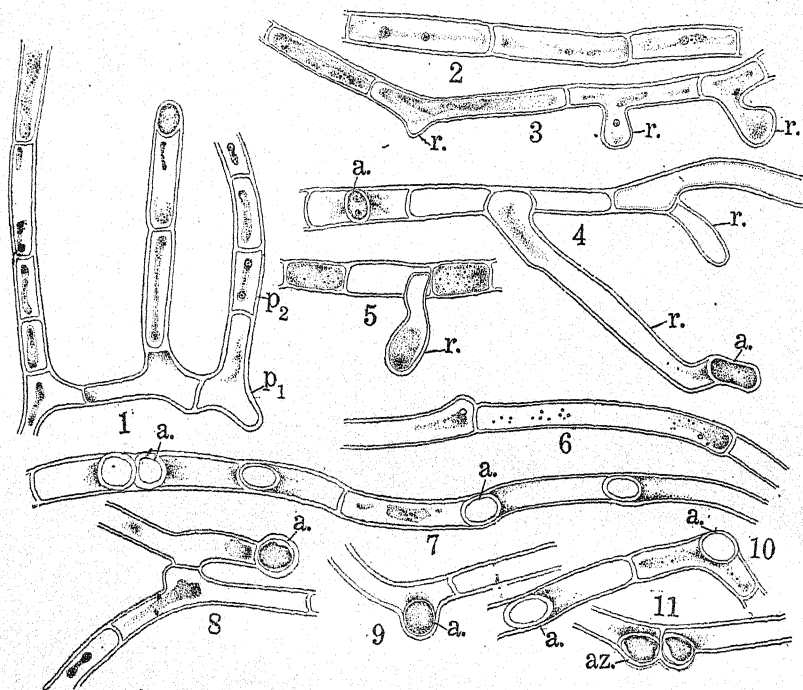
**Chloroplasts.**—The vegetative cells are 10–14  $\mu$  broad and 2–10 times as long. The product of assimilation consists of both oil and starch, oil preponderates and stains slightly black with osmic acid, while reaction with iodine is very slight. The chloroplasts are proportionately so small that they are visible only under very high magnifications and that also when the filaments are stained with an aqueous solution of Nile Blue. In most of the cells the chloroplasts are disorganised and only protoplasmic residue in the form of irregular plate-like bodies is found (Fig. 1). When first examined the alga appeared very enigmatic appearing more like a *Mougeotia* than a species of *Zygogonium*. The chloroplasts are small rounded bodies which may be close together or wide apart each bearing a small pyrenoid (Fig. 2). These rounded chloroplasts are easily disintiguishable from stellate chloroplasts of species of *Zygnema*. Unlike other species of *Zygogonium* cell-sap is not purple in this alga.

### Reproduction

This Himalayan alga is an aplanosporic form in which no conjugation lateral or scalariform has been observed. Only in one instance an abortive conjugation canal was observed (Fig. 8).

**Aplanospores.**—This alga reproduces mainly by means of aplanospores, which are ovoid bodies, 12–17  $\mu$  broad and 15–24  $\mu$  long. Aplanospores are usually formed at the ends of cells as in *Zygogonium capense* (Hodgetts) nov. comb. Transeau<sup>2</sup> (Fig. 7), and sometimes they may also be found centrally (Fig. 4) as in *Zygogonium hansgirgi* (Schmidle) nov. comb. Transeau.<sup>3</sup> Occasionally they are found terminally also (Figs. 1 and 8). They are usually produced

subaerially, but in many instances they are produced underground at the tips of rhizoids (Fig. 4). In such cases a migration of protoplasm takes place from the subaerial cell into rhizoids, and the protoplasmic matter accumulates at the lower end (Fig. 5), where it eventually rounds off, secretes a thick-cell-wall and develops into an aplanospore. Heavy deposit of cytoplasmic residue is seen in the cells surrounding the aplanospores on one side. Ripe aplanospores were not seen hence it is not possible to describe the sculpturing of the spore-wall.



Figs. 1-11. *Zygogonium kumaoensis* sp. nov.—Fig. 1. A plant showing prostrate ( $p_1$ ) and projecting ( $p_2$ ) portion. Fig. 2. A vegetative filament showing chloroplasts. Figs. 3, 4 and 5. Show different types of rhizoids ( $r$ ). Fig. 6. An early stage in the formation of aplanospores. Fig. 7. A chain of aplanospores ( $a$ ) in a filament. Fig. 8. An abortive conjugation canal. Figs. 9 and 10. Aplanospores ( $a$ ) in various positions. Fig. 11. Azygospores ( $az$ ). All ( $\times 980$ ).

Occasionally aplanospores may be seen apposed to one side of a cell which shows geniculation (Fig. 10). In some cases these aplanospores may be seen in swellings on the side touching the soil (Fig. 9). These resemble the azygospores described by Iyengar<sup>1</sup> in *Zygogonium talguppense*. One may as well consider these swellings as abbreviated rhizoids, though some would regard these as abortive conjugation processes, vestiges of an abandoned lateral mode of conjugation. Very rarely they may be found lodged in these

swellings in pairs cut off by a very thin wall from the other part of the cell as in *Z. talguppense* Iyengar (Fig. 2). Bodies found in this position are more appropriately described as azygospores, if we regard the swellings as abortive conjugation processes.

#### Affinities

Its peculiar chloroplasts and presence of cytoplasmic residue in cells, indicate that this is a species of *Zygogonium*. It differs from *Z. talguppense* Iyengar in its aplanosporic mode of reproduction and smaller size of its cells. The other related form is *Z. capense* (Hodgetts) nov. comb. Transeau from which it differs in its narrower and longer cells and varied position of its aplanospores. Hence it is desirable to describe this alga from Kumaon Himalayas as a new species which is described below.

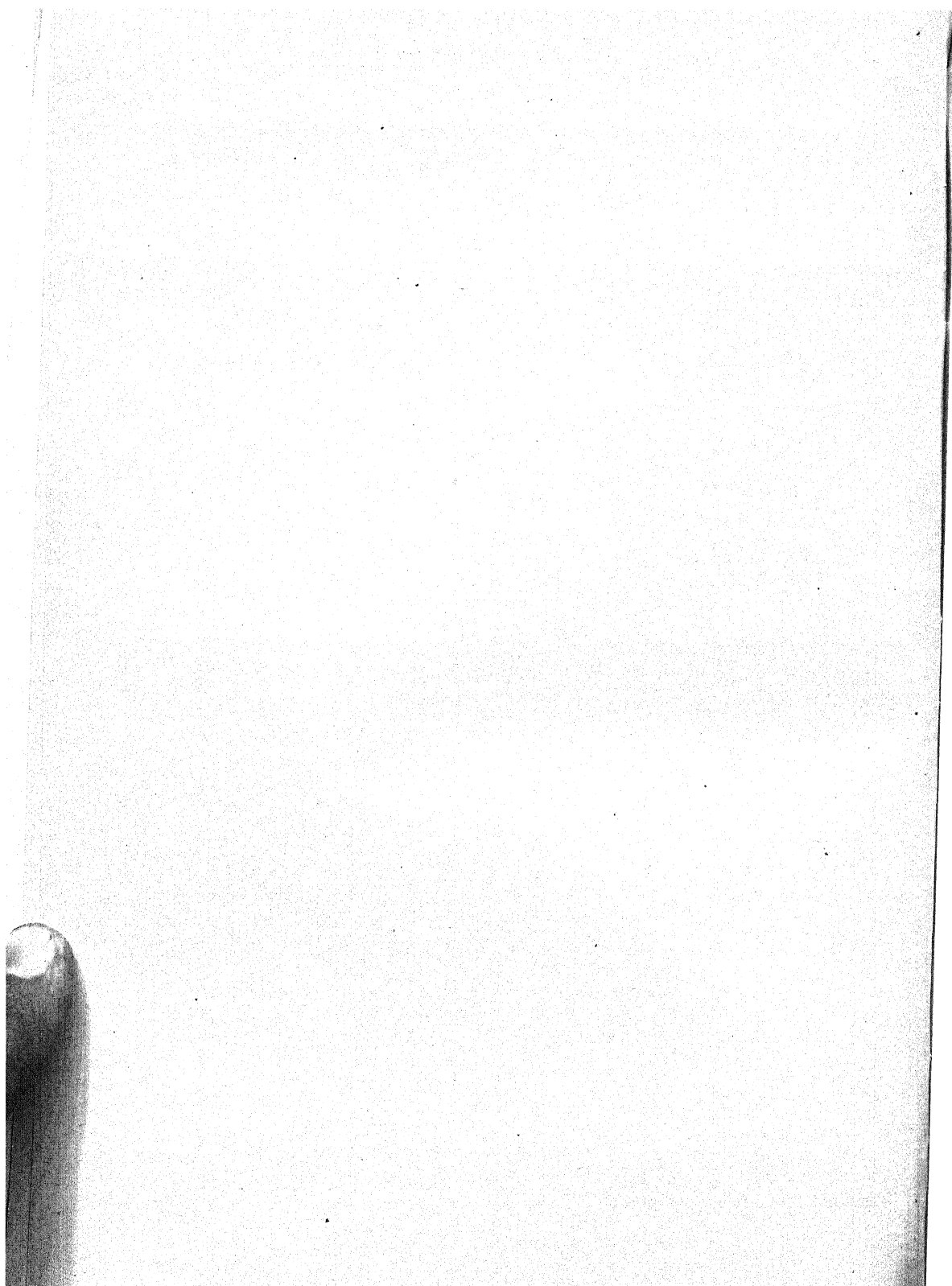
#### *Zygogonium kumaoensis* sp. nov.

Cellulis vegetativis 10–14  $\mu$  latis, 20–140  $\mu$  longis conjugatione incognitum. Generationibus per aplanosporis, globosis vel sub-globosis, 12–16  $\mu$  latis, 15–24  $\mu$  longis.

*Habitat.*—On rocks of red clay, Jariakhali, Almora, Kumaon Himalayas, India, September 1939.

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## STRUCTURE AND DEVELOPMENT OF SEED IN *SOPUBIA TRIFIDA* HAM.

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Received for publication on September 4, 1940

THE author has already studied the embryo-sac and endosperm haustoria of several members of Scrophularineæ (Krishna Iyengar, 1937; 1939 *a, b*; 1940 *a, b, c, d & e*), and during the course of these investigations an attempt has been made to classify these plants into various types (Author, 1940 *a*) according to the character of the haustoria.

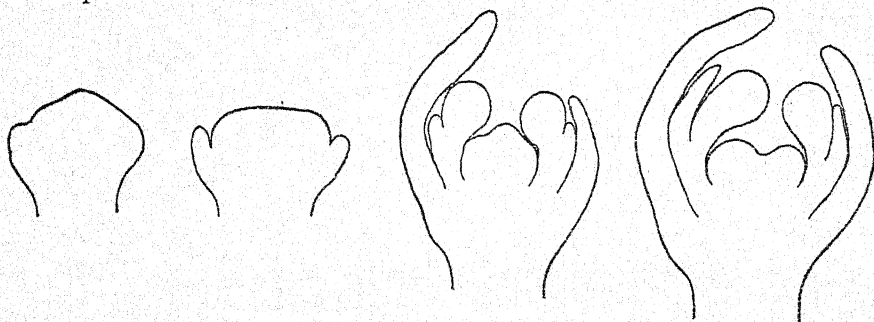
The first paper of the series (Author, 1937) gives a detailed description of *Sopubia delphinifolia* and the present species happens to be the second parasitic member of the family studied by me. Many other parasitic members of the family have been studied and described by various investigators. These are *Alectoreolophus*, *Euphrasia*, *Melampyrum*, *Pedicularis* (Balicka-Iwanowska, 1899; Schmid, 1906), *Striga* (Mitchell, 1915), *Tozzia* (Schmid, 1906) and others. Such works give an opportunity for studying several problems like the influence of parasitism on embryo-sac development, tapetal structure and endosperm-haustoria, and the influence of similarity of habitat on the embryogeny of closely related plants.

### MATERIAL AND METHODS

The material for investigation was collected from the hills round about Agumbe in the Western Ghats and fixed in Bouin's fluid. The sections were cut 8 microns thick for embryo-sacs and 10 microns for endosperm development. All the sections were stained in Heidenhain's iron-alum hæmatoxylin.

### ORGANOGENY OF THE FLOWER

Figs. 1*a, b, c* and *d* show the sequence in the development of the floral parts which is as follows: sepals, stamens, petals and carpels.



Figs. 1*a, b, c*, and *d*. Stages in the development of floral parts.

1 *a* ×185

1 *b* ×260

1 *c* ×130

1 *d* ×130



## OVARY AND OVULE

The ovary consists of a massive placenta with indefinite number of ovules arranged on it. The ovules consist of a reduced nucellus and a single thick integument. The placental cells are filled with starch grains; and rich protoplasmic contents characterise the cells of the integument. It was also noticed that there is a greater accumulation of starch in the placenta during post-fertilization stages than before.

## EMBRYO-SAC

The nucellus is composed of a large hypodermal cell surrounded by a layer of epidermal cells (Figs. 3 and 4). Even before the organization of the nucellus the pollen mother-cells have undergone the meiotic divisions. Fig. 2 represents the diakinesis stage in one of these cells. Nine bivalents were counted in this species although the  $n$  number for *S. delphinifolia* (Author, 1937) happens to be 18.

The hypodermal archesporial cell directly functions as the megaspore mother-cell. The formation of more than one archesporial cell noticed occasionally in the other parasitic genera was never met with in the two species of *Sopubia* studied by me. While the pollen tetrads are being formed, the archesporial cell enlarges and the integument makes its appearance (Fig. 3). With the subsequent development of the integument and the nucellus, the ovules assume an anatropous form (Fig. 4). A linear tetrad of megaspores (Fig. 5) develops from the megaspore mother-cell by two divisions taking place in quick succession. A normal eight-nucleate embryo-sac (Fig. 7) develops later on from the enlarging fertile megaspore towards the chalaza, while the other three degenerate and disorganize (Fig. 6). Just as in the other members the embryo-sac shows a dilated micropylar part, and a narrow chalazal part with the three small antipodals, the two polar nuclei being present in the middle of the sac. In the mature embryo-sac the protoplasm remains dense only in the neighbourhood of the egg-apparatus, polar nuclei and the antipodals. The egg-cell elongates and comes to lie very close to the two polar nuclei which fuse just before fertilization (Fig. 7).

From the two-nucleate stage of the embryo-sac onwards (Fig. 6), the nucellar epidermis begins to lose its contents and becomes almost flattened and crushed. At the micropylar end of the sac some of the cells of the nucellar jacket develop reticulate thickenings by the deposition of cellulose as shown in Fig. 8. The presence of these thickenings during the earlier stages and their absence during later stages is an interesting feature of this species.

## INTEGUMENT AND TAPETUM

The innermost layer of the integument develops into the tapetum. This is differentiated very early during the development of the ovule, and surrounds the entire nucellus. In the mature

embryo-sac only the non-dilated chalazal part of the sac is sheathed by the tapetum.

#### EMBRYO AND ENDOSPERM HAUSTORIA

Some stages in the development of the embryo have been studied by me and these are similar to those met with in other members of the family. The suspensor develops into a long tubular structure pushing the primary embryonal cell between the tiers of endosperm cells, and the latter begins to develop into an embryo only after the organization of a rich endosperm tissue.

The first division of the primary endosperm nucleus is followed by a transverse wall which divides the embryo-sac into a chalazal and an outer chamber (Fig. 9), the latter dividing again transversely resulting in a row of three cells (Fig. 10). The third division is longitudinal and takes place nearly simultaneously in all the cells (Fig. 11). The two middle cells develop into the body of endosperm by a series of transverse and longitudinal divisions, while the other cells form the haustoria. Just as in the other plants of the family previously investigated by me the two ends of the endosperm tissue are composed of smaller cells, very poor in starch but rich in protoplasmic contents. These in all probability assist in the transportation of nutrition from the haustoria to the more deeply placed endosperm tissue. The endosperm cells show abundant deposition of starch (Fig. 16).

#### ENDOSPERM HAUSTORIA

The micropylar tier develops into two haustorial cells. There is a nuclear division in each cell (Fig. 12). The partitional wall between the two cells is very thin and membranous, and disappears at a very early stage resulting in the formation of a highly aggressive tetra-nucleate body (Figs. 13 and 14), which continues to enlarge by digesting and absorbing the integumentary tissue in the neighbourhood of the vascular traces. There is a noticeable enlargement of haustorial nuclei, which stain very deeply during the later stages. Older haustoria show rich protoplasmic contents and a net-work of cellulose, the latter persisting till a very late stage of embryo and endosperm development (Fig. 17).

The two cells forming the chalazal tier develop into two simple uni-nucleate haustoria (Fig. 11). Just as in the micropylar haustoria, the thin separating wall disappears very early. This results in the formation of a single tubular bi-nucleate body (Fig. 14), which digests its way into the chalazal tissue and approaches the vascular tissue of the hilum. The formation of hypertrophied nuclei and the accumulation of rich protoplasmic material are common to both kinds of haustoria. The chalazal haustorium is the earlier to be formed as also the earlier to degenerate. With the degeneration of the haustoria the nuclei disintegrate into small bits and the haustorial walls show a slight increase in thickness by the deposition of cellulose. The haustorial action is so thorough that very

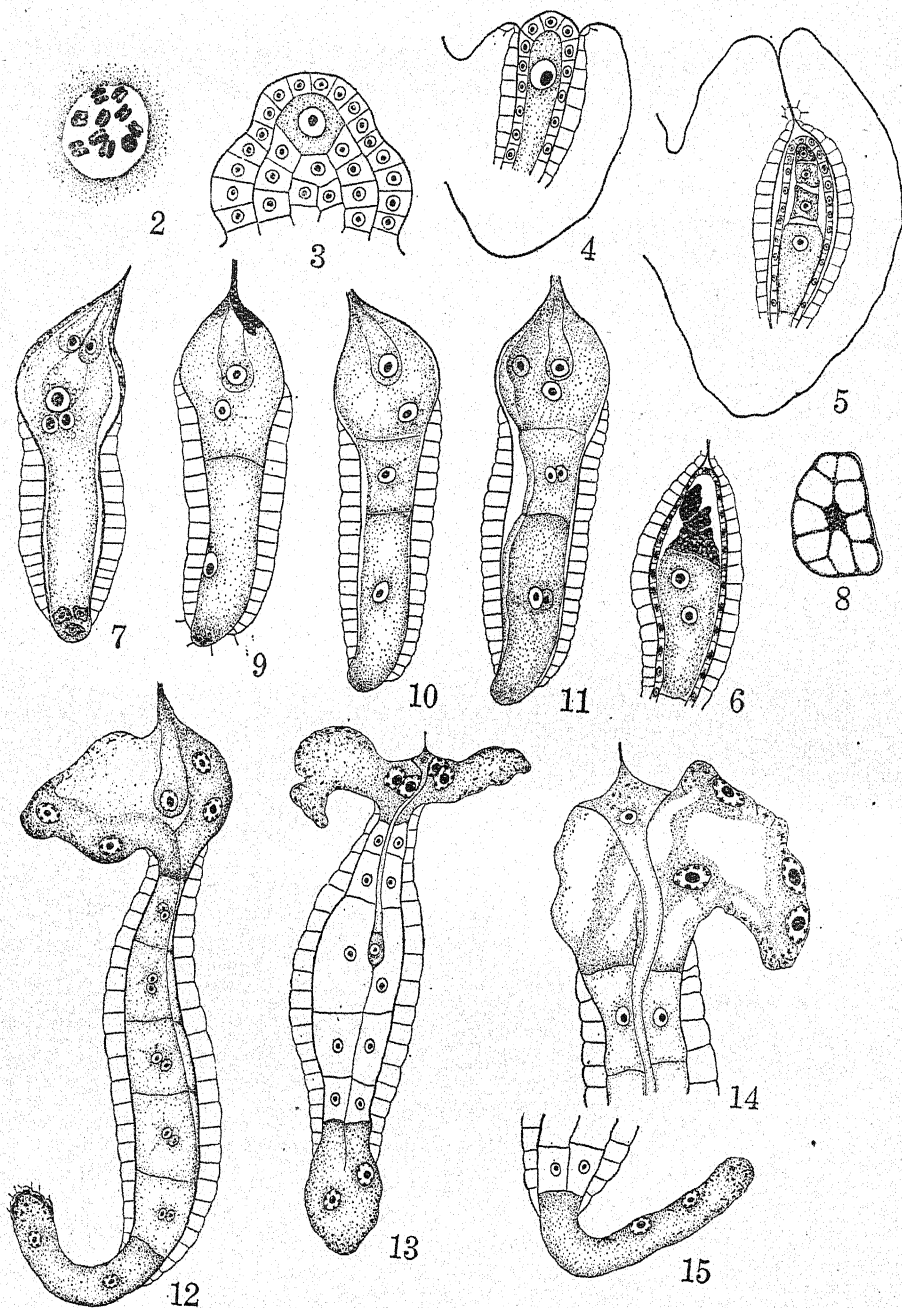
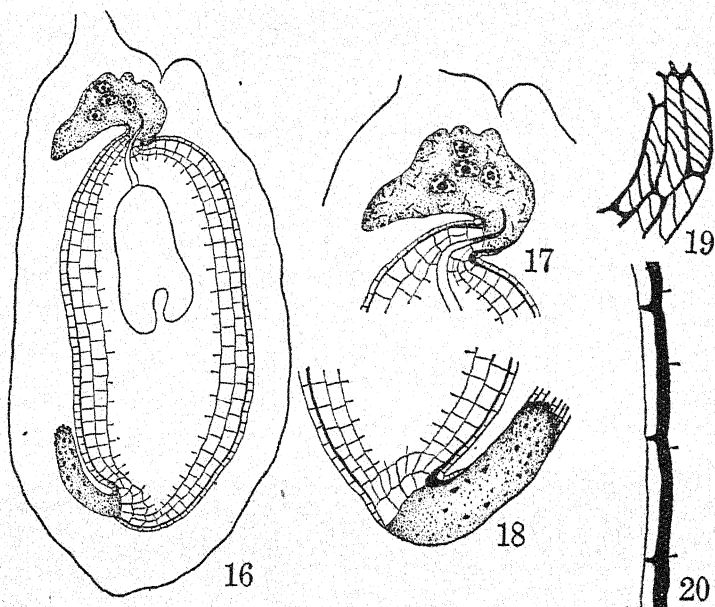


Fig. 2-15.—Fig. 2. Diakinesis in the microspore mother-cell.  $\times 1460$ .  
 Fig. 3. Nucellus and formation of integument.  $\times 640$ . Fig. 4. L. S.  
 of an ovule showing the integument.  $\times 320$ . Fig. 5. Linear tetrad

little tissue is left between the integumentary tapetum and epidermis. The tapetal cells lose most of their cell-contents, become reduced in size, but develop a thick cuticle towards the endosperm (Fig. 18).

#### FORMATION OF THE TESTA

As already mentioned, only the epidermis and the tapetal layer remain intact during later stages, although the latter shows marked



Figs. 16-20.—Fig. 16. L.S. of an almost mature seed showing fully formed embryo partly digested endosperm and the haustoria.  $\times 97.5$ . Fig. 17. Old micropylar haustorium with hypertrophied nuclei.  $\times 160$ . Fig. 18. Old chalazal haustorium with disintegrated nuclei.  $\times 160$ . Fig. 19. Scalariform thickening in the wall of epidermal cell.  $\times 65$ . Fig. 20. Epidermis of testa enlarged.  $\times 97.5$ .

reduction in the size of its cells. The walls of the epidermal cells become highly lignified (Figs. 19 and 20) indicating their protective rôle.

of megaspores.  $\times 480$ . Fig. 6. Degeneration of the three megaspores and the enlargement of the fourth.  $\times 480$ . Fig. 7. Fully formed embryo-sac.  $\times 480$ . Fig. 8. One of the nucellar jacket cells near the micropyle showing cellulose thickening.  $\times 1620$ . Fig. 9. First transverse division of endosperm.  $\times 320$ . Fig. 10. Second transverse division of endosperm.  $\times 320$ . Fig. 11. First longitudinal division of the three chambers.  $\times 320$ . Fig. 12. L.S. of the developing seed showing the two bi-nucleate micropylar haustoria, the two uni-nucleate chalazal haustoria and the two rows of endosperm cells.  $\times 320$ . Fig. 13. Slightly older stage than in Fig. 12. showing a single tetra-nucleate micropylar haustorium and a single bi-nucleate chalazal haustorium.  $\times 320$ . Fig. 14. Micropylar haustorium.  $\times 480$ . Fig. 15. Chalazal haustorium.  $\times 320$ .

## DISCUSSION

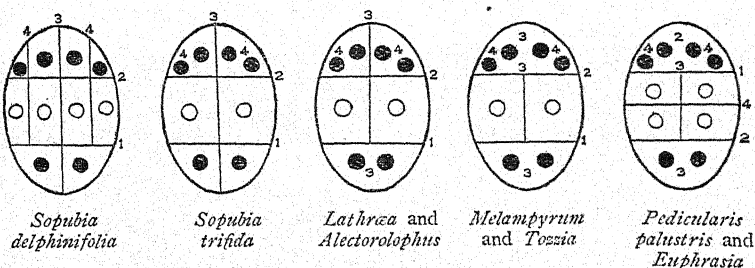
**EMBRYO-SAC.**—In all the members studied by me so far the embryo-sac develops from the innermost megaspore of the linear tetrad. While an embryo-sac with narrow tapering chalazal part containing small antipodals is quite a common occurrence, there are instances like *Stemodia* (Author, 1939 *b*) and *Melampyrum* (Schmid, 1906) where the embryo-sac is enlarged towards the chalaza. The former species is specially noted for the loosely arranged antipodal cells, while the latter is wanting in the antipodals when the embryo-sac is mature. The short and stout nature of the embryo-sac in *Melampyrum* is attributed to the resistance offered by elaborately formed cellular strands developing from the micropylar region of the integument and growing towards the interior. The antipodals are often small and three in number although occasionally only two (one of these bi-nucleate) may be present as in *Alectorolophus* (Schmid, 1906).

**INTEGUMENTARY TAPETUM.**—During the tetrad formation and early stages of the embryo-sac development the tapetum sheathes the nucellus completely. Only when the embryo-sac is reaching the eight-nucleate stage the nucellar jacket disintegrates, thus bringing the sac directly in contact with the tapetum. Exceptions to this are *Isoplexis* (Author, 1939 *a*), *Sopubia trifida*, *Melampyrum*, *Alectorolophus*, etc., where the nucellar jacket is present even when the sac is almost mature. *Melampyrum* (Schmid, 1906) goes a step further inasmuch as there are cellular integumentary strands developing from the micropylar region into the interior and almost blocking the micropyle. Although Hofmeister (1855) and Balicka-Iwanowska (1899) explain them to be tapetal outgrowths, Schmid (1906) feels that they are of a different origin. As regards their nutritional value there is no controversy. While tapetum often lines the non-dilated part of the embryo-sac, there are instances like *Alectorolophus* and *Melampyrum* where the entire sac is sheathed, and *Pedicularis* (Schmid, 1906) where the varying grades in sheath formation are noticeable.

The structure and development of tapetum and its nutritional relationship to the embryo-sac before and after fertilization have been described by several investigators. In *Celsia* (Author, 1939 *a*) *Vandellia* (Author, 1940 *a*), *Torenia* (Author, 1940 *c*), *Tetranema*, (Author, 1940 *d*) and others the tapetum shows the formation of large cells. It is also noticed that the significant development of these cells takes place at a time when the haustoria are very much reduced or begin to show senility. A digestive, absorptive and storage as well as a protective function has been ascribed to the tapetal layer, the enlargement of its cells and the peculiar thickening of its cell-walls lending further support to this view. There are many parasitic and non-parasitic members where the tapetum is of no great importance during the post fertilization stages of the embryo-sac development. Here instead of tapetal cells, the epidermis shows various kinds of thickening of the wall to meet the

mechanical requirements. Oil globules present in the epidermis of several members during the formation of seed are probably a device to meet the local demands.

**ENDOSPERM AND ENDOSPERM-HAUSTORIA**—The sequence of the earlier divisions in the endosperm is of great significance in the classification of the members into the different types. An attempt has already been made in this line by Glišić (1936-37), but some of the plants studied by me do not fit into any of the types described by him and have, therefore, to be treated separately. In all the cases the first two divisions are transverse. The third division is longitudinal, either extending to all the three chambers (*Sopubia*) or to the micropylar and middle chambers only (*Alectorolophus* and *Lathræa*) or only to the middle chamber (*Pedicularis*, *Euphrasia*, *Tozzia* and *Melampyrum*). *Lathræa* is remarkable for variation in the sequence of divisions. In this case at times the second division is longitudinal and the third is transverse, thus indicating some delay in the organization of the haustoria. This makes one suspect that nutritional factor may be responsible for this peculiarity. A second longitudinal division takes place in *Sopubia delphinifolia* but it is wanting in *S. trifida* and other parasitic members of the family. Here, only a nuclear division takes place. In *Pedicularis* and *Euphrasia* the micropylar and chalazal chambers show only nuclear division.



Diagrams illustrating sequence of wall formation in the earlier development of endosperm in some members of the group Rhinanthaceae

The haustorial structures can be arranged in a progressive series as in the accompanying diagram. *Sopubia delphinifolia* is least specialized, since it has in the beginning four uni-nucleate micropylar haustoria and two uni-nucleate chalazal haustoria, reminding one of the *Prolimosella*-type of Glišić (1936-37). This condition is also met with in *Herpestis* (Srinath, 1934), *Alonsoa* (Author, 1937), *Plysanthes*, *Bonnaya* (Author, 1940 b) and others. The next is *S. trifida* with two bi-nucleate micropylar haustoria and two uni-nucleate chalazal haustoria. The third stage, i.e., the occurrence of two bi-nucleate micropylar haustoria and one bi-nucleate chalazal haustorium is seen in *Alectorolophus* and *Lathræa* (Schmid, 1906). The last, i.e., the formation of a tetra-nucleate micropylar haustorium and a bi-nucleate chalazal haustorium right from the commencement is seen in *Pedicularis* (Schmid, 1906).



The older stages in all these members are alike inasmuch as they all possess a tetra-nucleate micropylar haustorium and a bi-nucleate chalazal haustorium. The thin separating membrane present between the haustorial cells towards the micropyle of *Sopubia* is completely absorbed, while in *Alectorolophus* there is partial dissolution. Thus the presence of a tetra-nucleate micropylar haustorium and bi-nucleate chalazal haustorium either from the commencement or during later stages is a fairly constant feature for all parasitic members of this family. In the non-parasitic members, on the other hand, there is considerable variation with regard to the number of haustoria and their nuclei. The greatest reduction in the number is seen in *Gratiola* (Glišić, 1933) and *Paulownia* (Millsaps, 1936). While the lateral haustorial branches or lobes are quite characteristic of the parasites dealt with above, it cannot be stated that this is always associated with parasitism, since in *Aeginetia* (Juliano, 1935) a parasitic member of the closely related family Orobanchaceae the micropylar haustorium is reported to be a non-functional body, and even the chalazal haustorium is not elaborate in its development. It may also be mentioned that even non-parasitic members like *Alonsoa* (Author, 1937), *Nemophila* (Svensson, 1925) and others show haustorial branches which are equally or even more elaborate than in the parasites.

In all the members of the Scrophulariaceae studied so far the first division of the primary endosperm nucleus separates a chalazal haustorial initial, although, judging from the statements of Schmid (1906), in a few cases like *Pedicularis* and *Euphrasia* the micropylar haustorium seems to be first formed. The next division is either transverse or longitudinal, the former resulting in the early formation of the micropylar chamber and haustorium, while the latter delays the organization of the same. This delay may be explained as due to some peculiarities in the nutritional conditions. Most of the members show the micropylar haustoria in a functional condition long after the chalazal ones have degenerated. Thus the chalazal haustorium is generally the first to be organized as also the first to degenerate.

Often one type of haustorium grows much more than the other. Even in a single type the different lobes show different grades in the development. This is very well illustrated by *Melampyrum* (Balicka-Iwanowska, 1899; Schmid, 1906) in which one of the two micropylar haustorial lobes is very well developed and grows into a long filiform body towards the hilum, while the other is very much reduced in size.

The older haustoria show hypertrophied nuclei, which stain very deeply in later stages. Along with this there are other changes also, like the deposition of cellulose either uniformly on the haustorial walls or in the form of rods or net in the haustoria. In *Pedicularis* the net-like appearance is quite a significant feature. A mechanical rôle has been ascribed to this structure, but the disappearance of the cellulose rods in the chalazal haustorium of *Vandellia hirsuta* suggests also the possibility of a nutritional rôle.

## SUMMARY

1. The ovule consists of poorly developed nucellus and a single massive integument. The hypodermal archesporial cell functions directly as the megaspore mother-cell.

2. A linear tetrad of megaspores is formed, the innermost cell developing further into the normal eight-nucleate embryo-sac.

3. An integumentary tapetum is organized at an early stage, but this lines only the non-dilated part of the mature embryo-sac, although during earlier stages the entire embryo-sac is surrounded by it.

4. The primary endosperm nucleus divides transversely to form two cells of which the upper again divides resulting in a row of three chambers, the chalazal, the middle and the micropylar. The next division is longitudinal resulting in two rows of three cells each. The two central cells develop into the body of the endosperm.

5. The chalazal cells form a single bi-nucleate haustorium by an early dissolution of the thin separating membrane.

6. The two micropylar cells enlarge and their nuclei divide resulting in two bi-nucleate haustorial cells which later coalesce to form a lobed and highly aggressive tetra-nucleate body growing towards the hilum.

7. In older haustoria the walls become thickened and the nuclei become highly chromatic and hypertrophied.

8. The endosperm shows two distinct regions—i.e., of smaller cells adjacent to the two haustoria and the other of larger cells situated in the middle. The smaller cells are richly protoplasmic and their chief function is probably to transport food materials from the haustoria to the more deeply situated tissue of the endosperm.

## ACKNOWLEDGMENTS

The author acknowledges his indebtedness to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M. (Chicago), University Professor of Botany, Central College, Bangalore, for the facilities for this investigation, and to Dr. P. Maheshwari, D.Sc., F.A.Sc., F.N.I., of Dacca University, for his kind perusal of the manuscript and helpful criticism.

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## THE SIGNIFICANCE OF ANATOMICAL CHANGES ACCOMPANYING REGENERATION OF X-RAYED BRYOPHYLLUM LEAVES

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Received for publication on July 18, 1940

### INTRODUCTION

THE problem of the effect of X-rays upon regenerating animal tissue, as widely separated as those obtained from coelenterates, flatworms, segmented worms, and chordates, has greatly attracted the attention of biologists during the last 40 years or so. The use of these rays has been considered in these investigations as important as that of stains or reagents in biological study made for disclosing many observational changes. Workers in this field like Zawarzin (1929), Curtis (1936), Bardeen and Baetjer (1904), Stone (1932), Litschko (1934) and Butler (1933) have most often concluded that the regenerative processes by which the missing parts become restored are usually retarded or completely inhibited according to the degree of exposure to the rays. It seems that such changes result as a sequence of irradiation of active cells which produce new tissues by division, migration and differentiation.

As a result of mutilation, regeneration in animals has been found to involve both histological and organismal changes. But our knowledge with regard to the effect of X-radiation in plant-regenerates is yet meagre. Freeland (1933) and Naylor (1940) investigated but the morphological aspect of *Bryophyllum* plantlets.

Although both organismal and histological factors are so inextricably mingled that they may be taken as different aspects of a unified series of events, yet of the two, the latter is likely to be more clearly recognised in so far as it consists of morphological and regional changes in cells. Moreover such a study of progeny plantlets does not seem to be made so far. In the present work, therefore, an attempt has been made to study in detail the induced anatomical modifications attendant upon irradiation of *Bryophyllum* leaves. Attempt has also been made to find out the nature and origin of regenerative or formative cells responsible for the production of plantlets.

## PROCEDURE

For experimentation eighteen *Bryophyllum calycinum* leaves of uniform size and shape were selected from plants of nearly the same age. Each of the leaves was approximately 7 cm. long with 20-22 marginal notches. In all including control, 6 sets of experiments were arranged and in each case 3 leaves were employed. The selection of leaves for individual experiments was made at random. The set-up of the tube used in the work was the following :—

Voltage ..	..	54 K.V.
Tube current ..	..	5 ma.
Distance ..	..	15 cm.
Anticathode ..	..	tungsten.
Exposure ..	..	2, 5, 10, 20, and 35 mins.

According to the above-mentioned exposures, 5 sets were irradiated. After treatment, leaves were kept in distilled water in suitable containers until in each case regeneration was complete. Daily records were made of the progress of regeneration. At successful intervals of plantlet growth, sketches were made to bring out the differences between control and irradiated progeny. For anatomical study the material was fixed with formalin-acetic-alcohol (Rawlins, 1933) from which  $10\mu$  thick sections were cut and subjected to detailed microscopical examination.

The chlorophyll content of leaves belonging to plant-lets from differently treated series, was determined by the recent method developed in this laboratory (Singh and Rao, 1937); the principle essentially consists in the measurement of the amount of light absorption of an alcoholic extract (80 per cent. methyl alcohol) of plant pigments within a narrowly defined region of the spectrum for which the chlorophyll ( $\alpha + \beta$ ) possess a marked absorption, while the absorption of the other pigments is infinitesimal.

## FINDINGS

*Morphological Changes :—*

Unlike Naylor's findings, regeneration in leaves treated for 2 minutes duration started on the 7th day, *i.e.*, 2 days earlier than the control set where buds at the notches showed sprouts on the 9th day. They were superior to the control as regards their rate of growth, number of leaves produced and length and number of roots (Fig. 1, *cf.* A and B). Each plantlet in the case of 2 minute-exposure possessed nearly 7 roots whereas the control could only produce 5 during the same period. The plantlets rapidly increased in size and attained the maximum height earlier than the control. The plantlets produced after 5 minutes treatment were much similar to those obtained from the untreated set with regard to almost all the characters noted above (Fig. 1, C). Still longer exposures caused retardation in these respects so that plantlets produced after 10 minutes irradiation had only 3 leaves after a lapse of 16 days (Fig. 1, D) when the untreated set was able to produce 6 leaves.

Greater retardation effect was noticed after 20 minutes treatment. In this case regeneration was markedly delayed and the plantlets

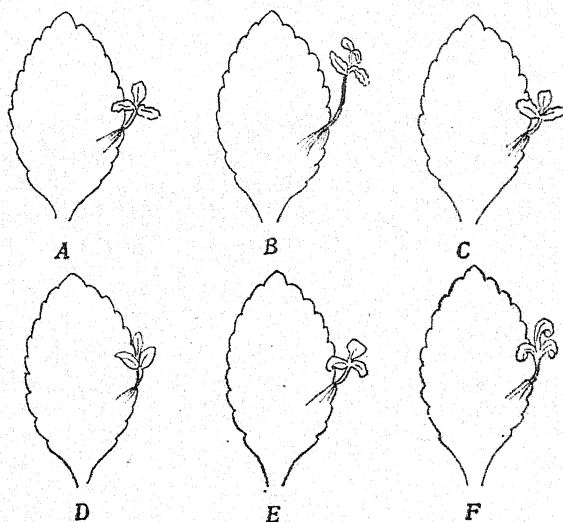


Fig. 1. *Bryophyllum calycinum* leaves with regenerates following different doses of X-radiation. The first three diagrams were made 16 days after treatment: A—control, B—2 mins. irradiated, and C—5 mins. irradiated.

Other three diagrams show the 3-leaf stage obtained after:

27 days in D (10 mins. irradiated)

39 " " E (20 " " )

47 " " F (35 " " )

could come to the 3-leaf stage after a duration of 26 days and even then the number of roots was smaller than in any of the previous sets (Fig. 1, E).

Unlike the effectiveness of the above-noted treatments a 35 minutes dosage gave some interesting observations. Regeneration in this case commenced as late as 21 days and the progeny showed marked morphological abnormalities. Such plantlets were obviously yellowish green in colour while an irregular distribution of light-yellow patches characteristically presented a mosaic appearance. Such observations following longer exposures have not been reported by Naylor (1940). Moreover the leaves belonging to these plantlets were usually irregular, often rolled and had an entire margin without any notches (Fig. 1, F). These observations are in close accord to the previous findings of Singh and associates (1939) in case of cotton seedlings raised from X-rayed seeds. Following irradiation the chlorophyll disturbances of the type noted above were also reported by Stadler (1930) in barley, by Morgan (1932) in Freesia, and by Wolcott (1936) in barley and castor plants.

*Anatomical Changes :—*

Under heavier doses (10 mins. and beyond) of X-radiation, the regenerative changes were inhibited in proportion to the duration of exposure. Naturally, therefore, the cell forms were greatly affected; but among the treatments utilised in this investigation the maximum variations were initiated by a 35 minutes dose. It is for this reason that special stress has been laid in this work to the study of regenerates belonging to this particular set.

*Leaf anatomy.*—Apart from the two epidermal layers and the ramifying veins, the bulk of control leaf is composed of large parenchymatous cells abundantly supplied with chloroplasts. There is practically no differentiation of mesophyll tissue into palisade and spongy parenchyma. The leaf structure as a whole is bilaterally symmetrical (Figs. 2, A).

Irradiation tends to modify the outline of the leaf. The central portion usually bulges out to form a raised structure (Fig. 2, B). The epidermal cells become turgid often assuming an irregular appearance. The mesophyll of the control leaf consists of thin-walled irregularly arranged compact cells with a few or no intercellular spaces, while the leaf tissue of irradiated plantlets showed loose arrangement of cells with numerous intercellular spaces (Fig. 2, cf. A and B). Unlike that of control the mesophyll tissue was pale, with groups of cells either having few plastids or none at all. Some of the cells showed even tissue distortion. In the control leaf-tissue there is a big central vascular bundle representing the midrib. This consists of large thick xylem vessels with loosely coiled spiral element of protoxylem at the extreme upper edge of the wood. Phloem vessels are close and small in size. Following irradiation the single vascular bundle divides into two dissimilar elements (one large and another small) where xylem cells are small though numerous and closely packed. Phloem vessels are much reduced in size and in number and are loosely arranged (Fig. 2, B).

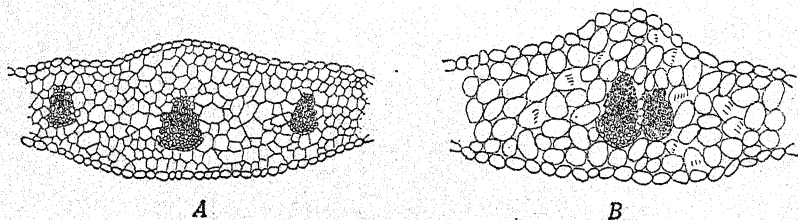


Fig. 2. Camera lucida drawings of the T. S. of leaf from different regenerates: A—control, B—35 mins. irradiated ( $\times$  ca. 100).

*Root anatomy.*—The cortical portion of the control sample consists of 8-10 concentric layers of polyhedral parenchymatous cells. In the irradiated roots this region contains a heterogeneous mixture of different sized cells which being loosely arranged increase the number and dimensions of inter-cellular spaces (Fig. 3, A and B).



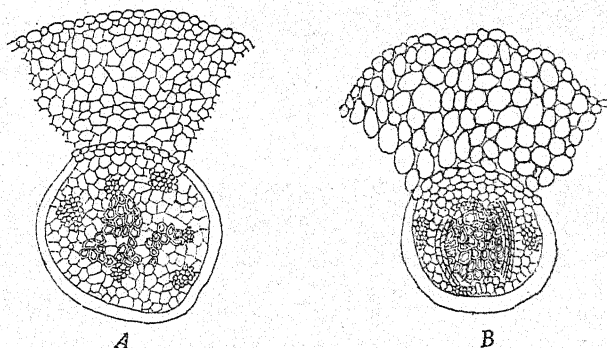


Fig. 3. Camera lucida drawings of the T. S. of root from different regenerates: A—control, B.—35 mins. irradiated ( $\times$  ca. 100).

More marked changes are observed in the stelar system of irradiated and unirradiated roots. The primary root in case of control is as usual a tri-exarch protosteles where the protoxylem element is composed of elongated spiral and annular vessels. The centrally placed meta-xylem region consists of large porous cells. The latter structure is far more developed in irradiated roots so that more than two-thirds of the vascular cylinder consists of meta-xylem while a few protoxylem cells are present but at the ends (Fig. 3, B). Such cells are thick and closely packed together. Alternating with the protoxylem points there are three groups of phloem separated by conjunctive tissue, which unlike that of control set become meristematic in roots of irradiated plantlets. The curved stripes of cambium thus found in the treated progeny, however, do not come in contact with protoxylem groups.

*Chlorophyll content.*—Slight changes in the colour of leaves of irradiated plantlets of short duration series became more marked as the duration of exposure increased. The greatest differentiation in leaf colour accompanied by variegated patches after a 35 minutes exposure gave stimulus to make study in this direction. The data on pigment analysis of different representative series have been shown in Table I.

TABLE I

*Chlorophyll content (in mgm. per 10 grams of fresh material) in control and X-rayed leaves of Bryophyllum regenerates*

Material	Chlorophyll content
Control	14.00
2 mins. treated	13.25
5    "    "	12.13
10   "   "	10.24
20   "   "	8.30
35   "   "	6.10



It is, thus, evident that there was a progressive decrease in chlorophyll accumulations as the duration of irradiation dose increased. Under 35 minutes treatment the chlorophyll content was lowered to even more than half obtained in the control set; being 6.10 against 14.0 mgm.

#### DISCUSSION

Naylor (1940) has reported suppression in regeneration with higher doses of X-rays but he does not mention any conspicuous changes in the regenerates of the type noted in this work. Following a half hour exposure, he obtained a marked suppression in the developmental activity of the mother tissue. In the present work, however, even a 35 minutes dose did not stop regeneration though the process was greatly delayed and the plantlets produced were invariably characterised with marked abnormalities.\* A two-minutes treatment, on the contrary, causes some accelerating effect upon regeneration. Observations of this nature also, accompanying short duration treatments, have not been noted by Naylor though Zawarzin (1929) working on *Pelmatohydra oligactis* concluded that shorter exposures accelerate regeneration. Similarly, activation following short duration radium treatment has also been reported by Congdon (1912) in case of *Tubularia crocea*. The variations in results of the present enquiry to those of Naylor may possibly be due either to the varietal difference in the types investigated or to the difference in the 'set-up' of the tube used for generating X-rays. The observations in general, however, lead to more or less similar conclusions that regeneration is retarded more and more as the duration of exposure to the rays increases. There is every likelihood that beyond a certain specific limit regeneration may even cease. Probably, a 35-minutes exposure initiating pale mosaiced leaves have very nearly approached the cessation limit.

The powerful action of X-rays is evident from the observations on plantlets obtained particularly after long duration treatments. Inhibition in growth, change in symmetry of leaves, and their characteristic coloration are evident examples. It has been noticed that as the duration of dose increases, the decrease in chlorophyll content becomes rapid. The slowness in the rate of growth of regenerates may then be accounted to a loss in the accumulation of chlorophyll, a factor associated with the process of photosynthesis.

The injurious action of X-rays is further visible on microscopical examination of leaf tissue where mesophyll shows distortion of cells and destruction of chloroplastids following irradiation. The cells of this tissue also become loose having numerous intercellular spaces in contrast to the close and compact cell arrangement observed in control samples. Increased leaf thickness and cell disintegration probably account for an increase in the inter-cellular spaces. This is

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\* In *Bryophyllum* the buds are present in the notches in a dormant state. Here one is really dealing with the effect of X-ray on growth.—Ed.

followed by a reduction in phloem bundles. Xylem element, on the contrary, is more developed both in leaf as well as in the root. These observations being in close accord to those of Miege and Coupe (1914), Johnson (1926), Wolcott (1936), and Singh *et al.* (1939); give some clue to the nature of cells responsible during regeneration. Freeland (1933) observes regarding the nature of cells responsible during regeneration, that stem and leaf primordia begin to grow first in proliferation and are exogenous in origin, while root-development is endogenous in both *B. calycinum* and *B. crenatum*.

Like previous observations of this Experiment Station [Singh, Choudhri and Kapoor (1939)], the irradiated leaf tissue in these investigations also contains two adjacent middle bundles instead of one present in the control. From the appearance of these dissimilar bundles placed side by side, one would conclude that the smaller of these is but an isolation effect of one whole indicating further that the midrib undergoes splitting during irradiation. As already pointed out these changes induced in regenerates obtained from X-rayed stock are more or less of a similar nature as those noted in cotton seedlings raised from X-rayed seeds. The seeds enclose radicle and plumule representing the embryonic root and shoot respectively. Following the treatment of seeds, therefore, it is assumed that the changes observed in seedlings develop from changes initiated in the embryo. Since the changes in the present enquiry exhibit a more or less similar trend, the development of plantlets from mother stock may easily be taken as embryonic rather than meristematic, that is to say, the plantlet is already organised in the notches. This agrees with the finding of Freeland (1933). It is anticipated that irradiation of meristematic tissue, may produce local changes, but will ordinarily not lead to such conspicuous plantlet abnormalities. Evidently then, it appears reasonable to conclude that there is embryonic structure present at each notch of the leaf, and it is this that is responsible for regeneration in *Bryophyllum*. This idea finds further support in the well-known law of Bergonie and Tribondeau (Curtis, 1936) wherein it is indicated that embryonic cells are far more susceptible to X-rays than the differentiated ones.

As evidenced, if regeneration involves the presence of cells having embryonic potencies, it may be supposed that the inhibition of regeneration or a change in the character of regenerates (morphological or anatomical) after irradiation results from injury or destruction of these embryonic cells without which regeneration cannot occur.

#### SUMMARY AND CONCLUSIONS

*Bryophyllum calycinum* leaves were exposed at notches to X-rays for durations lasting 2, 5, 10, 20 and 35 minutes and regeneration initiated by keeping the lower end of these leaves under water. Both morphological and anatomical observations were gathered, but in particular, changes in the latter aspect were accounted to

understand the nature of cells responsible during regeneration. The conclusions arrived at are :

Two minutes exposure had a slight stimulating effect but further higher doses inhibit regeneration.

Irradiation of leaves for 20 minutes caused some modifications in plantlets which became more distinct after longer exposure to X-rays.

After 35 minutes treatment leaves became asymmetrical, thick, rolled, had entire margin and presented a mosaic appearance. Chlorophyll content of the leaves decreased as the exposure to the rays increased.

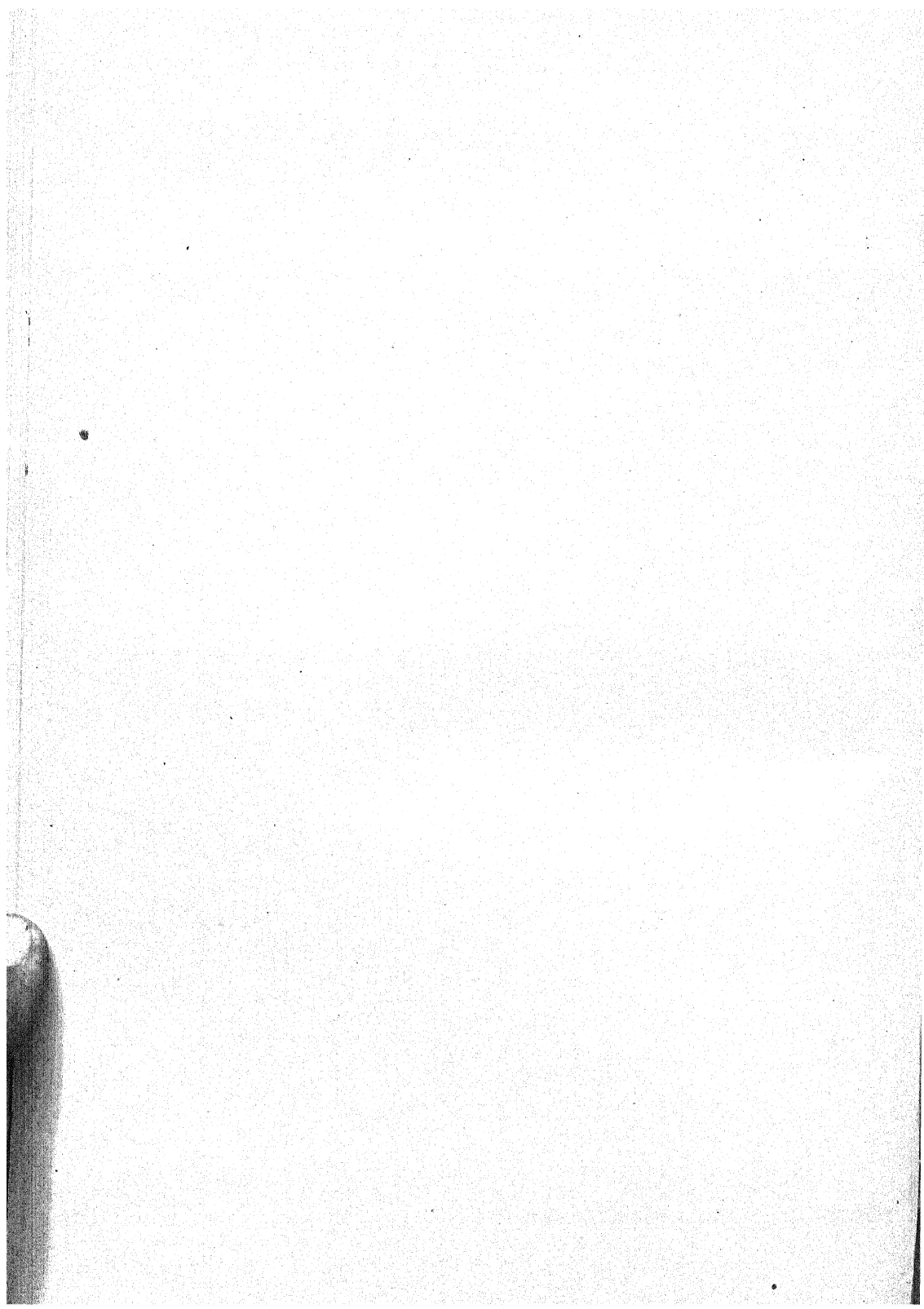
Structural changes were observed both in the leaves and in roots. The leaf mesophyll became loose and colourless indicating the presence of more air-spaces and a lack in the development of chlorophyll. The mid-rib splitted into two. Irradiation further activated development of xylem element but phloem was adversely affected. Root system of the irradiated plantlets showed greater development of metaxylem.

On the basis of anatomical changes it is assumed that the cells responsible for regeneration possess embryonic potencies.

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## STRUCTURE AND DEVELOPMENT OF THE EMBRYO-SAC OF *DRIMIOPSIS KIRKI* BAKER AND *ALLIUM GOVANIANUM* WALL.

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(Communicated by A. C. Joshi)

Received for publication on October 9, 1940

THE present paper describes the structure and development of the ovule and embryo-sac of two members of the Liliaceæ, *Drimiopsis kirki* Baker and *Allium Govanianum* Wall. The former is a native of tropical Africa, but is now grown in many tropical gardens. It bears somewhat globular flowers in spikes which are devoid of any bract-like involucre. *Allium Govanianum* is a small herb growing in the alpine Himalayas, with an underground bulb, narrow linear leaves and white flowers aggregated in involucre umbels at the summit of acutely angular scapes.

The material of both the plants was collected by Dr. A. C. Joshi. *Allium Govanianum* was obtained from near Khilanmarg (height 11,000–12,000 ft.) in the Kashmir State. The flowers were fixed in Navashin's fluid with a pretreatment in Carnoy's. The material of *Drimiopsis kirki* was collected from the Royal Botanic Gardens of Peradenyia (Ceylon) and Calcutta. It was fixed in formalin-acetic-alcohol and Karpetschenko's fluid. Customary methods of dehydration and embedding were followed and Heidenhain's iron-alum hæmatoxylin was used for staining.

### PREVIOUS WORK

The embryological literature bearing on the family Liliaceæ up to the year 1930 has been summarized by Schnarf (1931). Recently Joshi (1939) has summarized the important contributions which have appeared since then. In the present paper, therefore, only the literature bearing on the two genera *Drimiopsis* and *Allium* is mentioned.

The genus *Drimiopsis* has been studied so far only by Baranow (1926), who made a cytological and embryological study of *Drimiopsis maculata*.

The history of embryological work on *Allium* dates back to 1861, when Hofmeister described the mature embryo-sac, fertilization and embryo formation in *A. odorum*. The credit of the discovery of the bisporic type of embryo-sac development in *Allium* goes to Strasburger (1879), whose results were later confirmed by Fischer (1880) and Schniewind-Theis (1901). Strasburger (1879) also

described the egg-apparatus and discussed the homologies of the several nuclei in the embryo-sac. The formation of embryos from antipodals was reported in *A. odorum* by Tretjakow (1895) and Hegelmaier (1897). According to them only one antipodal cell, which shows certain correspondence with the egg cell, invariably develops into the embryo, but occasionally embryos are also formed from other antipodals also. The occurrence of synergid-embryony was also recorded. Hegelmaier (1897) noted the presence of adventitious embryos in some of the seeds examined by him.

Among the later workers, mention may be made of Schürhoff (1923) and Haberlandt (1922, 1923). The former studied the phenomenon of fertilization in *A. odorum*. According to him all the adventitious embryos are not formed from the antipodals, an observation which was not confirmed by Haberlandt. Modilewski (1925) reinvestigated *A. odorum* and tried to offer an explanation from the cytological view-point of adventitious embryo development. Schürhoff (1926) has given an extensive summary of the cytoembryological investigations on this genus up to 1925.

Weber (1929) investigated several species of *Allium* and her work is a substantial addition to the previous literature. She observed several types of synergids, the early degeneration of antipodals, the fusion of polar nuclei before fertilization and lastly the presence of supernumerary nuclei in the pollen tubes of *Allium rotundum*.

Messeri (1931) studied the embryo-sac development in six species of *Allium*. In *A. neapolitanum* he observed the absence of antipodals and all the four chalazal nuclei functioning as polar nuclei. He also recorded antipodals functioning as eggs in *A. nigrum*, *A. subhirsutum* and *A. schoenoprasum*. Modilewski (1931) described the development of the embryo in *A. odorum*. Maheshwari (1937) has listed the species of which the embryo-sac has been studied so far.

#### DRIMIOPSIS KIRKI

(a) *The Structure of gynoecium and the ovule.*—The gynoecium is tricarpeal, syncarpous. The superior ovary is trilocular and shows two anatropous ovules in each loculus. The ovules are collateral so that a longitudinal section (Fig. 1) shows only one and a transverse section (Fig. 2) two ovules in each loculus. The ovules are erect, bitegmic, with the micropyle pointing downwards and arise from near the base of the ovary (Fig. 1). The outer integument consists of 3-4 layers of cells, the inner of two layers through its greater length but of 3-4 layers close to the micropyle. The micropyle is formed only by the inner integument.

The development of the ovule is quite normal. The inner integument develops before the outer. When it has attained the height of the nucellus, the primordium of the outer integument also makes its appearance. The nucellus is poorly developed. At the tetrad stage it consists of 2-3 layers of cells, but 1-2 layers are



crushed by the growing embryo-sac, so that the mature embryo-sac is practically covered by the epidermis alone (Fig. 3).

One of the interesting features of the ovules is the development of a hypostase-like strand of cells in the chalazal region of the nucellus connecting the tubular antipodal region of the embryo-sac with the vascular bundle of the ovule (Fig. 3). The cells of this strand are regularly arranged and are densely filled with cytoplasm. The presence of such a strand was noted by Wunderlich (1938) in *Yucca filamentosa* and by Joshi (1939 and 1940) in *Iphigenia indica*, *Gloriosa superba* and *Gagea fascicularis*. It appears to be a general feature of the family and serves the purpose of conducting food material to the embryo-sac.

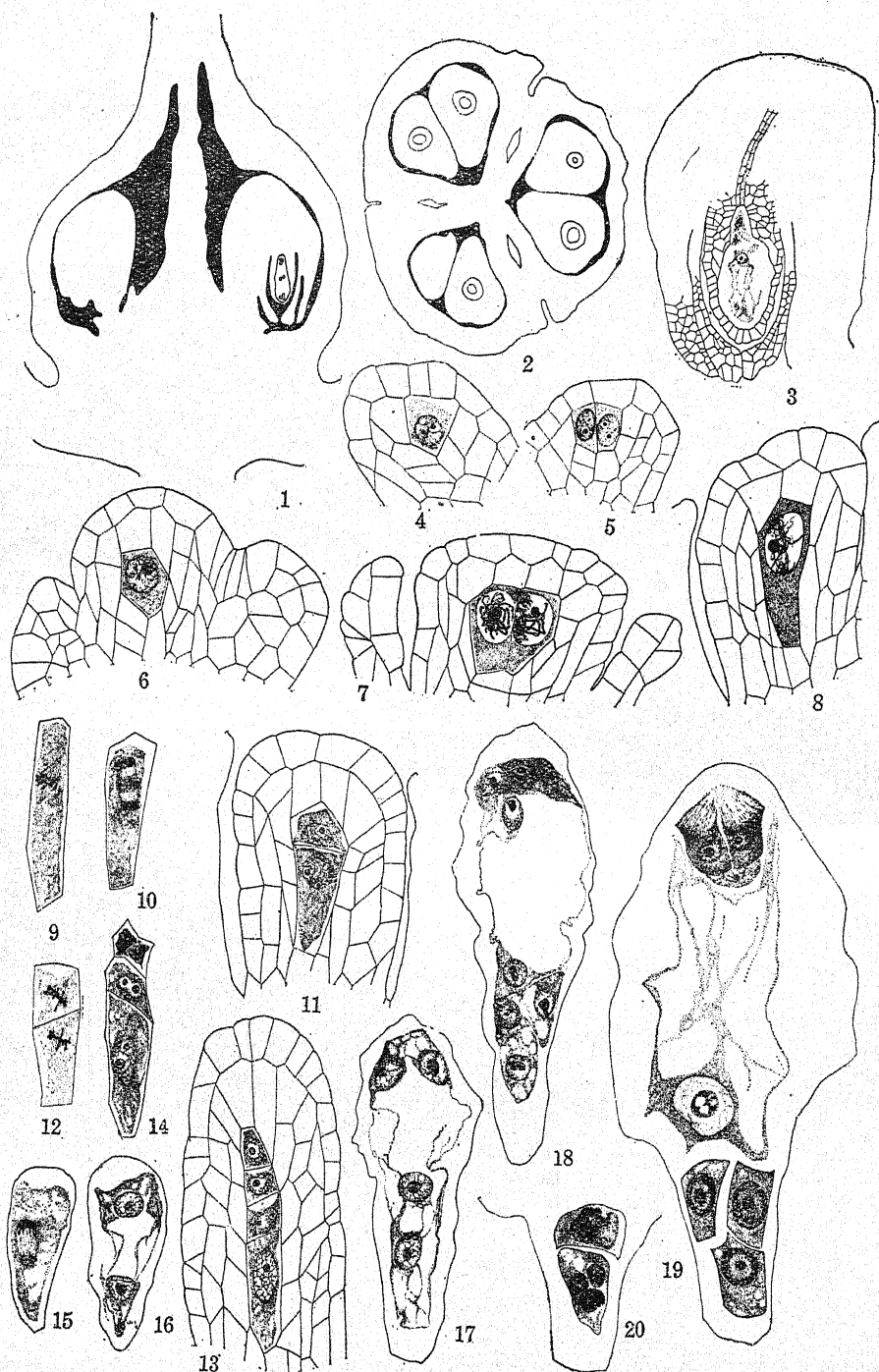
(b) *Development of the Embryo-sac.*—The number of primary archesporial cells varies from one to three. These differentiate rather early, before the primordia of the integuments make their appearance (Figs. 4 and 5). The occurrence of more than one archesporial cells appears to be a general variation in the family Liliaceæ, several examples being cited by Schnarf (1931), such as *Gagea lutea*, *Lilium Martagon*, *Lilium candidum*, *Lilium longiflorum*, *Lilium philadelphicum*, *Fritillaria messanensis*, *Fritillaria meleagris*, *Veltheimia* sp., *Yucca gloriosa*, etc. Each archesporial cell cuts off a parietal cell (Figs. 6 and 7), which never divides periclinally, so that only a single layer of parietal cells is formed. This is later crushed by the growing embryo-sac.

Only one archesporial cell develops beyond the megaspore-mother-cell stage. The functional megaspore-mother-cell elongates considerably, undergoes the two meiotic divisions (Figs. 8–12) and forms a row of four megaspores (Fig. 12). Sometimes the orientation of the spindle during the second division in the upper dyad cell is oblique and a T-shaped tetrad is the result (Fig. 14), as reported by Joshi (1939) in *Iphigenia indica*, Maheshwari and Singh (1930) in *Asphodelous tenuifolius* and by Watkins (1938) in *Yucca rupicola*.

The chalazal megaspore always develops into the embryo-sac. It enlarges, becomes vacuolate and appears as the young gametophyte. The degeneration of the remaining megaspores starts from the micropylar end. Where a T-shaped tetrad occurs, the upper two megaspores are the first to degenerate. Vacuolation continues in the uni-nucleate gametophyte and the nucleus now enters the first mitotic division (Fig. 15), as a result of which two nuclei are formed. These nuclei are pushed to the opposite poles by the growing central vacuole (Fig. 16).

The 2-nucleate embryo-sac gives rise to the 8-nucleate stage in the normal manner (Figs. 17 and 18). During its development the embryo-sac expands on all sides, but particularly at the micropylar end. The cytoplasm aggregates at the two poles and these are connected by fine strands of cytoplasm. The antipodals are organised before the egg-apparatus (Fig. 18).





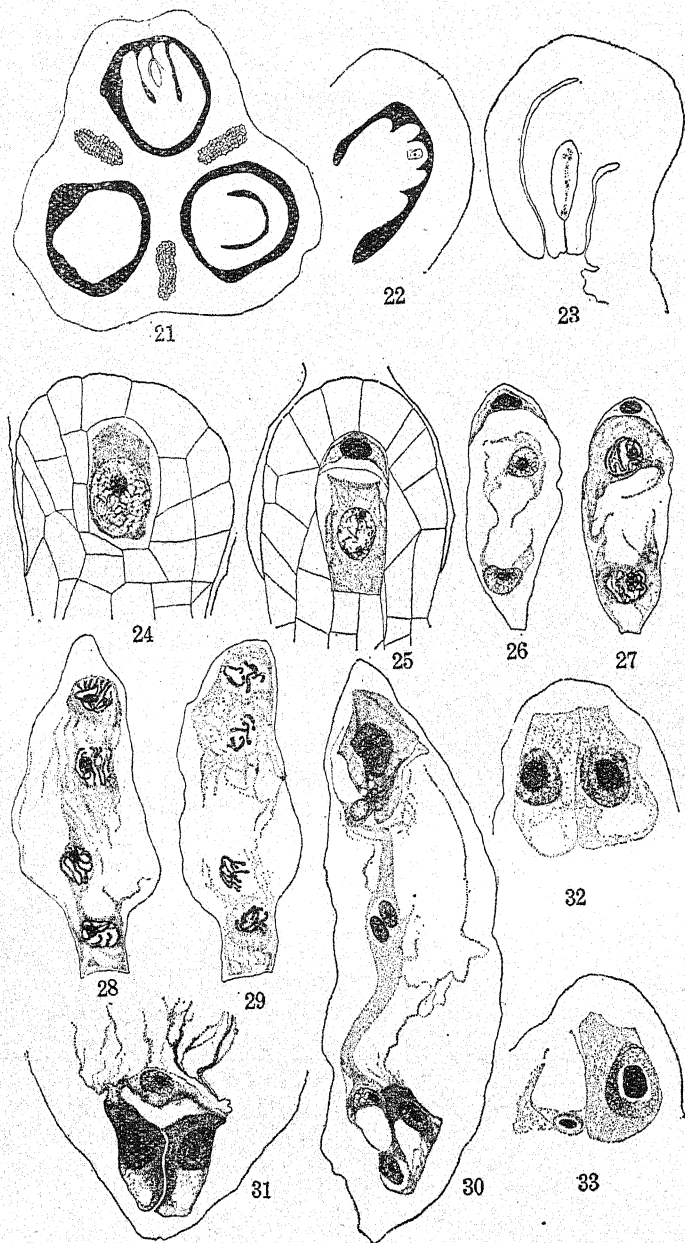
Figs. 1-20. *Drimiopsis kirki*.—Fig. 1. Longitudinal section of the ovary showing the form and arrangement of the ovules. Fig. 2. Transverse section of the ovary showing its tricarpeal nature and two ovules

(c) *The mature embryo-sac.*—The mature embryo-sac has a bulbous form with a narrow tubular chalazal portion (Fig. 19). The egg has got the usual structure, but its cytoplasm stains rather lightly and appears hyaline. The synergids have a beak-like apex and are provided with a distinct 'filiform-apparatus' as previously noted in *Yucca* (Habermann, 1906), etc. For the full list reference may be made to the paper by Dahlgren (1928). Another peculiarity of the synergids is the total absence of any vacuoles. They are densely filled with cytoplasm. The same feature has also been noted by Joshi (1939) in *Iphigenia* and by Watkins (1937) in *Yucca*. It appears to be wide-spread in the family. In favourable sections hooks are visible on the synergids. The nucleus is situated below the hook. The antipodals are found in the narrow chalazal portion of the embryo-sac. They are generally uni-nucleate, but in one case an antipodal with three nuclei was also observed (Fig. 20). Joshi (1939) has listed the Liliaceæ which show multi-nucleate antipodals. The polar nuclei meet in the middle of the embryo-sac, but the secondary nucleus formed by their fusion moves to the antipodal end of the embryo-sac. This indicates that the endosperm in this genus also most probably develops according to the *Helobiales*-type, which Wunderlich (1937) has found to be characteristic of the Scilloideæ, the tribe of the Liliaceæ to which *Drimiopsis* belongs.

#### ALLIUM GOVANIUM

(a) *Structure of the gynoecium and the ovule.*—Each loculus of the trilobular superior ovary has only one anatropous ovule (Figs. 21 and 22). The gynoecium of *Allium Govanianum* is thus considerably reduced as compared with other species of the genus. The ovule has two integuments, which are more massive than those of *Drimiopsis* and develop nearly simultaneously. The outer integument is 4-6-layered and the inner though its greater length 3-layered. The micropyle is long and narrow, and is formed only by the inner integument (Fig. 23). The nucellus is even more reduced than in *Drimiopsis*. At the dyad stage the micropylar apex shows only one layer of the nucellar cells, the epidermis of the nucellus (Fig. 25). Later even this is crushed and the embryo-sac touches the epidermis of the inner integument, which is from the very beginning in very close contact with the nucellus.

in each loculus. Fig. 3. Longitudinal section of the ovule showing the structure of the integuments, nucellus and hypostase-like strand. The ovule is nearly mature. Figs. 4-5. The primary archesporium. Fig. 6. Archesporial cell after cutting off the parietal cell. Fig. 7. Two megaspore-mother-cells in one ovule. Fig. 8. A megaspore-mother-cell. Figs. 9-10. The I meiotic division in the megaspore-mother-cell. Fig. 11. Dyad stage. Fig. 12. II meiotic division. Fig. 13. Tetrad of megaspores. Note the parietal cells which do not divide at all periclinally. Fig. 14. Early degeneration of the two micropylar megaspores. Fig. 15. First division in the embryo-sac. Fig. 16. 2-nucleate embryo-sac. Figs. 17-19. 4-nucleate, 8-nucleate and mature embryo-sac stages. Note the chalazal tube in the mature embryo-sac. Fig. 20. Multi-nucleate antipodals. Figs. 1, 2 and 5 ( $\times 175$ ). The rest ( $\times 800$ ).



Figs. 21-33. *Allium Govanianum*.—Fig. 21. Transverse section of the ovary showing one ovule in each of the three loculi. Fig. 22. Ovules at the primary archesporial stage. Only a part of the ovary is shown. Fig. 23. Anatropous ovule at the mature embryo-sac stage. Fig. 24. Primary archesporium. Fig. 25. Dyad showing the early degeneration

(b) *Development of the embryo-sac.*—About the time the primordia of the integuments are formed a single hypodermal cell differentiates as the primary archesporial cell (Fig. 24), which functions directly as the megaspore-mother-cell (Fig. 25), as noted by Strasburger (1879) in *A. fistulosum*, by Modilewski (1925) in *A. odorum*, and by Dahlgren (1927) in *A. strictum*. Dahlgren (1927) states that there is a wide variation in the formation of the parietal cell in the family Liliaceae. *Drimiopsis kirki* and *Allium Govanianum* investigated by the writer well illustrate this character.

The megaspore-mother-cell increases in size and divides to form two dyad cells (Fig. 25). The upper one of the two degenerates very soon (Figs. 26, 27), while the one towards the chalazal end develops into the embryo-sac. The development follows the *Scilla*-type (Figs. 26–30). The 2-nucleate stage is quite normal, but the 4-nucleate stage is characterised by the arrangement of the nuclei more or less in a row, with two at each pole. The position of the nuclei differs from that of *Drimiopsis kirki*, where the embryo-sac follows the *Normal*-type of development.

(c) *Mature embryo-sac.*—The mature embryo-sac is roughly fusi-form in shape (Fig. 30). The synergids are comparatively bigger than the egg. Strasburger (1879) distinguished in *Allium fistulosum* a large synergid occupying the summit of the embryo-sac by the side of the egg and a smaller one situated slightly below. This character was not noted in the present material. The synergids attain large size, and possess a large nucleus occupying more or less a central position. They show hook-like projections slightly above the middle. They may be full of dense cytoplasm without any vacuoles (Fig. 33), or show small vacuoles (Fig. 30) or a single large vacuole (Fig. 32) towards the chalazal end. No. 'filiform-apparatus' was noted. Weber (1929) working on several species of *Allium* also recorded different types of synergids, the significance of which she discussed with regard to fertilization. The egg is a flask-shaped structure, poor in cytoplasm and with a relatively small nucleus. The polar nuclei fuse in the centre. The fusion takes place before fertilization as noted by Strasburger (1879) in *A. fistulosum* and Weber (1929) in *A. paniculatum*. Of the three antipodals, one is conspicuous from the others (Fig. 31) by virtue of its position, by organisation of the internal cytoplasm and by the possession of a smaller nucleus like the egg. It shows a clear resemblance to the egg, although the other two antipodals do not show the structure of the synergids. Modilewski (1931) observed in *A. nigrum* and in *A. paniculatum* the antipodal cells exhibiting a close correspondence with egg-apparatus, the other antipodal

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of the micropylar cell. Figs. 26 and 27. 2-nucleate embryo-sacs. Figs. 28–29. Division stages (prophase and metaphase) of the 4-nucleate embryo-sac. Fig. 30. Mature embryo-sac. Fig. 31. Antipodals. Fig. 32. Synergids with chalazal vacuoles. Fig. 33. A synergid densely filled with cytoplasm and without vacuoles, and the egg. Figs. 21–23 ( $\times 175$ ). Figs. 24–33 ( $\times 1400$ ).

cells resembling synergids even in the possession of "filiform-apparatus". Messeri (1931) also recorded the same feature in three species studied by him. Further, this sort of organisation is not confined to the genus *Allium* alone in the family. According to Ernst (1902) in *Paris quadrifolia* and *Trillium grandiflorum* also the antipodals now and then assume the form of the egg-apparatus.

#### DISCUSSION

The family *Liliaceae* is remarkable in showing great variation in the development of the embryo-sac (Schnarf, 1931) and almost all types of development have been observed within this family. In *Drimiopsis kirki*, as in *Drimiopsis maculata* investigated by Baranow (1926), the embryo-sac has been found to develop according to the *Normal*-type. The only unusual features are the absence of vacuolation in the synergids, which as stated before has also been noticed in a number of other *Liliaceae*, and the occasional occurrence of multi-nucleate antipodals. The chalazal end of the embryo-sac shows tubular extension, in which are placed the antipodals. Reed (1903) and Watkins (1937) attribute a haustorial function to this chalazal tube. In the case of *Drimiopsis* also it appears to have the same function, as it penetrates into the strand of elongated cells connecting the chalazal end of the embryo-sac with the vascular bundle of the ovule ending in the chalaza. It is clear that most of the food material must be passing into the embryo-sac through this tubular chalazal end.

The embryo-sac in *Allium Govanianum* has been found to develop according to the *Scilla*-type, also called the *Allium*-type (Maheshwari, 1937). This mode of development appears to occur with remarkable uniformity in all the species of *Allium* investigated so far. Only Weber (1929) has noted that in *Allium paradoxum* the development follows the *Normal*-type. This author noted the presence of three tetrads, but as Maheshwari (1937) says she did not see all the developmental stages. The synergids of *Allium Govanianum* are variable in structure. They may be densely filled with cytoplasm or possess only small vacuoles or a single large vacuole. Still more interesting, however, is the organisation of an egg-like antipodal. In *Allium odorum* this feature has been found to be connected with the development of antipodal embryos (Hegelmaier, 1897). It may, therefore, be interesting to study the development of embryo in *Allium Govanianum* also.

The differentiation of one of the antipodals in several species of *Allium* as an egg and in some species of the three antipodals as an egg-apparatus supports the view of Porsch (1907), according to which the 8-nucleate embryo-sac of angiosperms is equivalent to two archegonia.

#### SUMMARY

The embryo-sac of *Drimiopsis kirki* develops according to the *Normal*-type. The mature embryo-sac is characterised by the

development of a chalazal haustorial tubular extension, the absence of vacuoles from the synergids, presence of 'filiform-apparatus' on the synergids, and the occasional presence of multi-nucleate antipodals. The number of primary archesporial cells varies from 1-3 and a parietal cell is cut off.

The embryo-sac of *Allium Govanianum* develops according to the *Scilla*-type. It is characterised by the variable structure of the synergids and antipodals. The single primary archesporial cell cuts off no parietal cell.

The writer takes this opportunity of expressing his sincere thanks to Dr. A. C. Joshi, of Benares Hindu University, under whose guidance this investigation has been carried, for suggesting the problem and supplying the necessary material.

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# A CONTRIBUTION TO THE LIFE-HISTORY OF *BERGIA CAPENSIS* LINN.

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Received for publication on September 26, 1940

## CONTENTS

	PAGE
I. INTRODUCTION .. .. .	283
II. MATERIAL AND METHODS .. .. .	283
III. OBSERVATIONS .. .. .	284
(a) Microsporogenesis .. .. .	284
(b) Megasporogenesis .. .. .	284
(c) Ontogeny of the Integument .. .. .	284
(d) Embryo .. .. .	286
(e) Endosperm .. .. .	288
IV. GENERAL CONSIDERATIONS .. .. .	288
V. SUMMARY .. .. .	289
VI. LITERATURE CITED .. .. .	290

## I. INTRODUCTION

THE small family of Elatinaceæ of the Parietales has only two genera, *Elatine* and *Bergia*, both of which are represented in India. Most of the previous work in the family is confined to the genus *Elatine*. Kajale (1940) has in a recent paper described the life-history of *Bergia ammanioides*, Roxb. Friesendahl (1927) has described the development of the embryo-sac in the other genus, i.e., *Elatine*. In the present paper, the life-history of another species of *Bergia*, namely *Bergia capensis*, is described. The haploid chromosome number of *Bergia capensis* has also been determined for the first time. The haploid chromosome numbers of only two other species of *Bergia* have been previously determined (Hagerup, 1932). Besides this, no other work seems to have been done in the genus *Bergia*.

## II. MATERIAL AND METHODS

*Bergia capensis* is a water plant rooting at the lower nodes. Flowers are borne in clusters in the axils of leaves. Material for the present investigation was collected from plants found growing in a rice field in Manalur. Anthers of the required stage were determined by acetocarmine examination and then fixed in Karpechenko's Navashin. Ovaries and flower buds were fixed in corrosive sublimate-formalin-acetic-alcohol and were embedded in paraffin in the usual way. Sections were cut at 6 to 10  $\mu$  thickness. Newton's



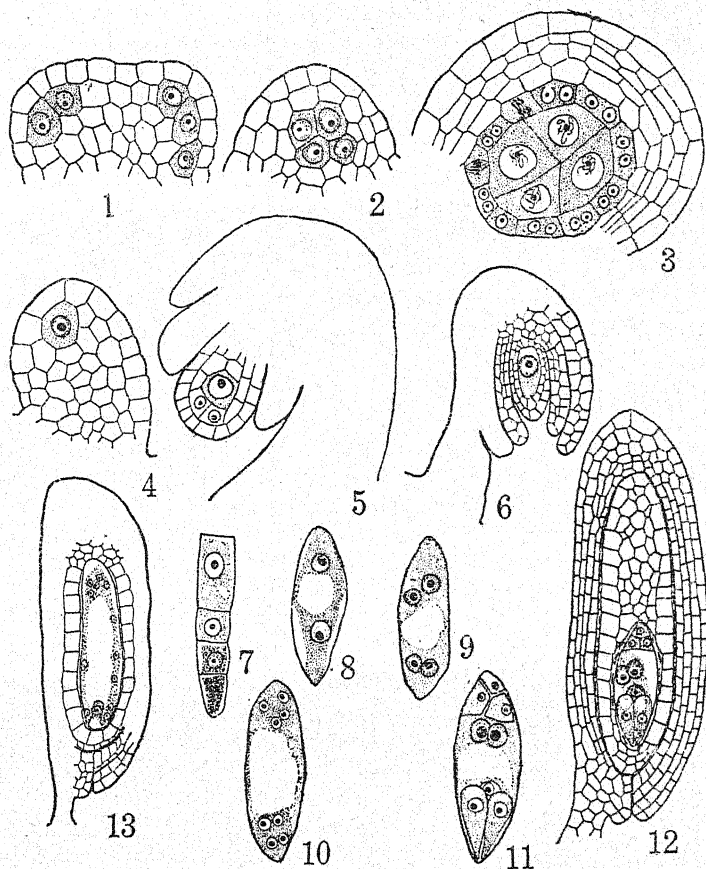
Iodine Gentian Violet was employed for cytological preparations while Heidenhain's Iron alum Hæmatoxylin was used for staining the embryo-sac and embryo.

### III. OBSERVATIONS

(a) *Microsporogenesis*.—A row of two or three hypodermal cells constitute the primary archesporium of the anther lobe (Fig. 1). These hypodermal cells cut off a layer of primary parietal cells (Fig. 2). This parietal layer divides periclinally into two layers. The two layers thus formed, again divide periclinally. As a result, four layers of wall cells excluding the epidermis are formed (Fig. 3). The innermost layer of wall cells becomes the tapetum, the hypodermal layer becomes the fibrous endothecium, while the two middle layers are gradually crushed as the anther develops. The tapetal cells to begin with, are uni-nucleate but during the prophase of the pollen mother-cells, become bi-nucleate and in this condition they remain till their final disorganization. Their division is mitotic in nature. Fig. 3 shows different stages in the mitotic division of the nucleus of the tapetal cells. Some workers have reported fragmentation or amitosis as the typical method of nuclear division of the tapetal cells. For example, the nuclei of the tapetal cells divide by amitosis in *Datura stramonium* (O'Neil, 1920). Bonnet (1912) and Cooper (1933) have found the tapetal nucleus dividing by ordinary mitosis. Cooper recognised three types of tapetal cells: (1) in which the tapetal cells remain uni-nucleate, (2) in which the tapetal cells become bi-nucleate and (3) in which the tapetal cells are pluri-nucleate. Similar mitotic division of the tapetal nuclei has also been recorded in *Gynandropsis* (Raghavan, 1938) and *Angelonia* (Raghavan and Srinivasan, 1940). The microspogenous cells after further mitotic division become the pollen mother-cells and round themselves off. At first metaphase nine bivalents can be counted in the pollen mother-cell (Fig. 14). The arrangement of the microspores is usually tetrahedral. The pollen grains at the time of shedding are two-celled (Fig. 15).

(b) *Megasporogenesis*.—The ovary is composed of five carpels with numerous anatropous ovules arranged along an axile placenta. Very early in the development of the ovule a hypodermal archesporial cell is differentiated (Fig. 4). It cuts off a parietal cell and functions as the megaspore mother-cell (Fig. 5). The primary wall cell undergoes first an anticlinal division and by further periclinial and radial divisions a comparatively massive parietal tissue is built. There is evidence to show that to its constitution the epidermal cells of the nucellus also contribute by their division. The nucellus on the sides of the mature embryo-sac is about two cells thick (Fig. 12). As the endosperm and the embryo begin to develop in the embryo-sac, these two layers of cells are crushed.

(c) *Ontogeny of the Integument*.—There are two integuments. The integuments arise as protuberances from the base of the ovule. The inner integument is the first to arise, the outer integument



Figs. 1-13. *Bergia capensis*.—Fig. 1. Hypodermal archesporial layer of an anther lobe ( $\times 1500$ ). Fig. 2. The archesporial cells have cut off a layer of primary parietal cells ( $\times 1500$ ). Fig. 3. Shows the five-layered anther wall, the innermost of which is the tapetum. Note the mitotic division of the tapetal nucleus ( $\times 1500$ ). Fig. 4. Primary archesporium of the ovule ( $\times 1500$ ). Fig. 5. Megaspore mother-cell after cutting off wall cell ( $\times 1100$ ). Fig. 6. Ovule showing three-layered inner integument and two-layered outer integument ( $\times 500$ ). Fig. 7. Linear tetrad of megaspores ( $\times 1500$ ). Figs. 8-11. Two-, four-, eight-nucleate and mature embryo-sacs ( $\times 1500$ ). Fig. 12. Ovule with eight-nucleate embryo-sac, showing the massive parietal tissue, and the degeneration of the innermost layer of cells of the inner integument ( $\times 500$ ). Fig. 13. Ovule showing free nuclear division of endosperm nucleus ( $\times 335$ ).

arising later. Both the integuments are composed of two layers of cells to begin with, but at about the time when the megaspore mother-cell is about to enter upon the meiotic phase, the inner integument becomes three layered, due to the division of the inner layer of cells (Fig. 6). At about the same time, the outer integument

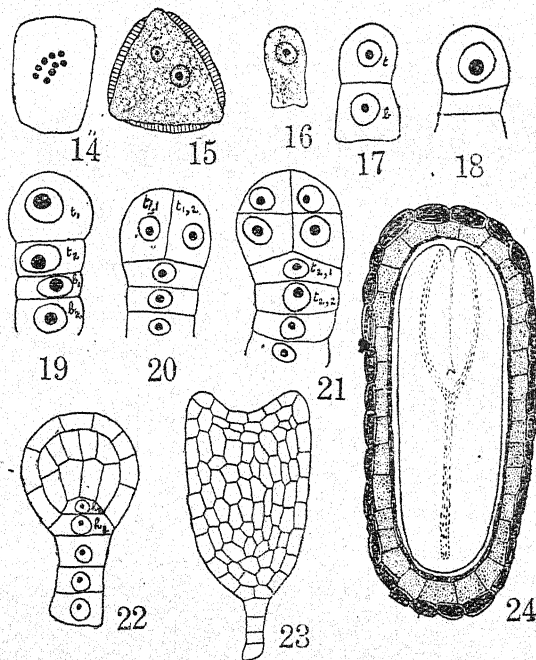
which was initiated later grows rapidly and overtakes the inner integument. The two halves of the inner integument meet above the tip of the nucellus and form the micropyle. The outer integumental halves growing rapidly meet above the inner integument. Both the integuments take part in the formation of the micropyle. At about the time when the embryo-sac in the ovule becomes eight-nucleate, the innermost layer of the inner integument begins to degenerate (Fig. 12). As the ovule matures into the seed, the inner layer of the outer integument and the middle layer of the inner integument degenerate. As a result, the testa in the mature seed is composed of the outer layer of the outer integument and the outer layer of the inner integument. The remains of the degenerated inner layer of cells of the inner integument can also be seen (Fig. 24).

The megaspore mother-cell increases in size and undergoes the reduction division as a result of which a linear tetrad of megaspores is formed (Fig. 7). Kajale (1940), however, found in *Bergia ammannioides* a T-shaped tetrad or a linear tetrad and in one instance a row of three megaspores (strictly speaking a dyad and two tetrads). The chalazal megaspore functions while the rest degenerate. The single nucleus divides and gives rise to a binucleate embryo-sac (Fig. 8). The two nuclei divide twice in succession to form an eight-nucleate embryo-sac (Figs. 9 and 10). In the eight-nucleate embryo-sac there are two groups of four-nuclei one towards each end of the embryo-sac (Fig. 10). The mature embryo-sac is comparatively small and elliptical in shape (Fig. 11). The two synergids are very prominent as also the polar nuclei and the antipodals. The polar nuclei fuse either in the middle of the embryo-sac or more towards the antipodal end (Fig. 11) and not towards the egg apparatus as is usually the case. All these eight cells occupy a large part of the space of embryo-sac (Fig. 11). The antipodals degenerate immediately after fertilization as also the synergids.

(d) *Embryo*.—After fertilization the oospore (Fig. 16) undergoes a period of rest at the end of which it divides transversely to give rise to a terminal cell (*t*) and a basal cell (*b*) (Fig. 17). These two cells again divide transversely and as a result a linear pro-embryo four cells long is formed ( $t_1, t_2, b_1, b_2$ , Fig. 19). The four-celled proembryo stage is a very important one, since each of these cells gives rise to a definite region in the mature embryo, and the method is usually the same in all members of the group or family. There are two types of arrangements of the cells of a four-celled proembryo. Either they may be arranged in a linear fashion as in the present case and as is common in the Rubiaceæ, Solanaceæ (Souegés, 1922, 24), Leguminosæ (Cooper, 1933; Weinstein, 1926) and Umbelliferæ (Borthwick, 1931) or the upper two cells may be one over the other, and the lower two cells lie side by side. This type of arrangement is recorded in Cruciferæ, Ranunculaceæ (Souegés, 1913, 19) and Capparidaceæ (Raghavan, 1937).

The terminal cell ( $t_1$ , Fig. 19) of the four-celled proembryo is responsible for the formation of the embryo proper. It divides

longitudinally to form a quadrant ( $t_{1,1}$ ,  $t_{1,2}$ , Fig. 20). Another division at right angles to the first, results in the formation of an octant (Fig. 21).



Figs. 14-24. *Bergia capensis*.—Fig. 14. Metaphase I showing nine bivalents ( $\times 1500$ ). Fig. 15. Mature two-celled pollen grain ( $\times 1100$ ). Figs. 16-23. Various stages in the development of the embryo. Explanation in the text. Figs. 16-21 ( $\times 1500$ ). Fig. 22 ( $\times 1100$ ). Fig. 23 ( $\times 750$ ). Fig. 24. Mature seed. The testa is composed of two layers of cells.

The second cell from the tip ( $t_2$  in Fig. 19) now divides transversely to form two cells ( $t_{2,1}$  and  $t_{2,2}$  in Fig. 21). The one towards the micropyle ( $t_{2,2}$ ) does not divide again but forms part of the suspensor. The other cell, which is next to the embryonal sphere ( $t_{2,1}$ ), functions as the hypophysis. The hypophysis divides transversely into  $h_1$  and  $h_2$ , the upper of the resulting two cells ( $h_1$  in Fig. 22) becomes continuous with the dermatogen, while the lower cell ( $h_2$  in Fig. 22) arches into the embryonal sphere and forms part of the periblem (Fig. 22).

The two remaining cells of the four-celled proembryo ( $b_1$  and  $b_2$  in Fig. 19) and the sister cell of the hypophysis ( $t_{2,2}$  in Fig. 21) forms the suspensor which is thus composed of three cells. Fig. 23 shows the lobing of the cotyledons. The mature embryo (Fig. 24) is straight with short and equal cotyledons. The two plerome strands of the cotyledons shown in Fig. 24 by dotted lines meet the plerome of the root at the hypocotyledonary region. The plumule

is enclosed between the cotyledons and appears as a small papillate protuberance. There appears to be no well-marked endosperm tissue surrounding the cotyledons.

(e) *Endosperm*.—The triple fusion nucleus divides repeatedly before the division of the oospore (Fig. 13) in a free nuclear fashion. The resulting free endosperm nuclei are distributed along a peripheral layer of cytoplasm in the embryo-sac, while a number of them are found accumulated in the chalazal part of the embryo-sac where the cytoplasm is rather dense. Wall formation which synchronises with the lobing of the cotyledons, commences from the micropylar region and extends to the chalazal part. In *Bergia ammanioides* (Kajale, 1940) also, it is so, while in *Elatine* (Friesendahl, 1927) wall formation in the endosperm tissue starts at the chalazal part of the embryo-sac and proceeds towards the micropylar part.

#### IV. GENERAL CONSIDERATIONS

A small family of the Parietales, the Elatinaceæ, consists of about 35 species distributed over two genera *Elatine* and *Bergia*. The basis of differentiation between the two genera is that while the genus *Elatine* is characterised by two- or four-merous floral parts, in the genus *Bergia*, the floral parts are five-merous. The chromosome numbers known so far in this family are very few. The chromosome numbers of only one species of *Elatine*, and three species of *Bergia* including *Bergia capensis* are known.

<i>Elatine hydropiper</i> n ..	20	<i>Bergia capensis</i> ..	9
		<i>Bergia ammanioides</i> ..	12
		<i>Bergia suffruticosum</i> ..	18

The chromosome numbers known in this small family are so few that any attempt at generalization in the direction of cyto-taxonomy may be premature. But the very circumscribed nature of the family, and the data already available make it possible for us to make a few pertinent observations in respect of the two genera comprising the family.

The relationship between chromosome numbers and systematic position has been definitely established in several cases and as such the value of chromosome numbers as a guide to taxonomy is now undoubted. In recent years attempts have been made to analyse on a chromosomal basis the taxonomic status and affinities of plant genera. Often the results of these attempts have confirmed the classification of the species made on the basis of their morphological characters. For example, Smith (1932) working on the cyto-taxonomy of the genus *Anchusa*, found that two species in the genus did not have the basic number of the genus and as such could not, on cytological grounds, be included in the genus. Johnston (quoted by Smith) purely on taxonomic grounds had removed the same two species to genera other than *Anchusa*. Thus the removal by Johnston of these two species on

purely taxonomic grounds, was confirmed on the criterion of cytological data also. Such coincidence of morphological and taxonomic features with cytological data, especially the chromosome numbers, makes it possible to use these as an additional basis for classification and very often as a verifying factor. In this small family there are only two genera and these would appear, upon cytological grounds, to conform to two series, *Elatine* following the 5-series and the genus *Bergia* following the 3-series. That there is sufficient ground for the differentiation of the two genera on the basis of floral characters, there can be no doubt and the chromosome numbers only confirm the distinctiveness of the two genera. These two differ from one another in such a fundamental character as the number of the floral parts. It may seem not unjustifiable to make these into two sub-families Elatinoideæ and Bergioideæ each being monogeneric. Very often we find that such a division of a family into sub-families is associated with a difference in the basic chromosome number of one or two. Recently we found (Raghavan and Srinivasan, 1940) that the division of the Aizoaceæ into the Molluginoideæ and Ficoideæ was correlated to a difference in basic number, 8 for Ficoideæ, and 9 for Molluginoideæ. These would indicate, however, that the primary basic number of the family would be a lower number and that these are to be regarded as secondary balances of the primary number. Indication of this is to be sought in secondary associations, that may be exhibited by the different members, and if possible, supported by genetical data. For example in the family Papaveraceæ (Sugiura, 1940), the Chelidonoideæ would conform to the 3-series, the Hypecoideæ to the 4-series and the genus *Papaver* is said to be the most highly developed genus amongst the Papaveraceæ both for anatomical and karyological reasons. Species of Papaveroideæ contain multiples of the sum of 3 and 4. These data would make us believe that further observations in this circumscribed family both as regards chromosome numbers and the prevalence of secondary associations, etc., will throw more light in support of the suggestion that has tentatively been made. It is also interesting to note that this differentiation is associated with a morphological detail which at first sight may appear unimportant. This is in respect of the method of wall formation in the endosperm tissue. In the genus *Bergia*, wall formation commences from the micropylar end of the embryo-sac, while in the genus *Elatine*, it commences from the chalazal part.

#### V. SUMMARY

1. The haploid chromosome number of *Bergia capensis* Linn. is nine.
2. The archesporium in each anther lobe consists of a row of two to three hypodermal cells. The development of the anther sac is described as also the behaviour of the tapetum.
3. The mature pollen grain at the time of shedding is two-celled.



4. The ovary is penta-carpellary. The ovules are anatropous and the placentation is axile. The origin and development of the integuments are described and correlated with the testa in the seed. Each ovule has two integuments, the outer two cells thick and the inner composed of three cells.

5. The development of the embryo-sac is found to be normal. Parietal tissue is rather massive and is built out of the primary parietal cell and also by the epidermal cells of the nucellus dividing.

6. Embryogeny is traced in some detail.

7. The endosperm development is free nuclear, wall formation starting from the micropylar end.

8. The importance of chromosome numbers in taxonomy is discussed. The family is viewed on cyto-taxonomical grounds, and it is found that both for cytological and morphological reasons the two genera comprising the family are quite distinct. A tentative suggestion for the division of the family into these two monogeneric sub-families is put forward. The scope for future work along these lines is also indicated.

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## STUDIES IN THE BIGNONIACEÆ

I. Chromosome Number and Epidermal Hydathodes in  
*Spathodea campanulata* Beauv.

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Received for publication on September 20, 1940

CONTENTS				PAGE
I.	INTRODUCTION	..	..	293
II.	MATERIAL AND METHODS	..	..	294
III.	OBSERVATIONS	..	..	294
	(a) Meiosis	..	..	294
	(b) The Secretion	..	..	295
	(c) The Trichome	..	..	296
IV.	GENERAL CONSIDERATIONS	..	..	297
V.	SUMMARY	..	..	298
VI.	LITERATURE CITED	..	..	298

## I. INTRODUCTION

MORPHOLOGICAL and cytological work on Bignoniaceæ is very meagre. The chromosome numbers recorded so far cover only the following ten species distributed over five genera.

<i>Campsis radicans</i>	..	..	..	$n = 20$
<i>C. grandiflora</i>	..	..	..	$n = 18$
<i>Incarvillea grandiflora</i>	..	..	..	$n = 9$
<i>I. Delavayi</i>	..	..	..	$n = 9$
<i>Tecoma capensis</i>	..	..	..	$n = 17$
<i>T. jasminoides</i>	..	..	..	$n = 19$
<i>T. Smithii</i>	..	..	..	$n = 18$
<i>T. Tagliabuana</i>	..	..	..	$n = 20$
<i>Catalpa syriacifolia</i>	..	..	..	$n = 20$
<i>Bignonia venusta</i>	..	..	..	$n = \text{ca } 25$

Mention has been made of this genus *Spathodea* mainly in connection with the occurrence of water-secreting glands. The earliest observations on these hydathodes were made by Treub (1889) in *Spathodea campanulata*. In addition to the scales, brown hairs covering the calyx, and the water secreting glandular hairs, there have been recorded also other sugar-secreting glands in this genus. In *Spathodea stipulata*, Rao (1927) noted the occurrence of sugar-secreting glands in addition to the usual nectary around the base of the

ovary. These glandular structures were found on all the floral parts sometimes scattered and sometimes forming local aggregations.

Our attention was drawn to this species on account of the prominent turgid flower buds. It is a medium sized garden tree said to be a native of South Africa. It flowers in our garden during the months of February and March. The haploid chromosome number of this species has been determined for the first time and a few observations made on meiosis; some details are also given of the ontogeny of the epidermal hydathodes as also an analysis of the fluid secreted by these glands.

## II. MATERIAL AND METHODS

The material for this investigation was collected from plants growing in the University Botanic Garden. Flower buds of the right stage of development were selected and a preliminary acetocarmine examination was made. This did not give a very satisfactory indication of the nuclear divisions. The copious presence of starch grains adds to the difficulty. But by judicious warming over an alcohol flame and by keeping the slide for a number of hours without drying, the chromosomes show themselves out somewhat clearly. Care should however be taken not to overheat the preparation. As this process entails such a long delay, the fixation of the anthers could not be made with any amount of precision as regards the meiotic stages. Aceto-carmine examination was made use of only to ensure that the pollen mother-cells had already been rounded off. Navashin's fluid with a slightly lower percentage of formalin than usual (about 6 per cent. less) was employed. Embedding was done in the usual manner with chloroform as the paraffin solvent. Sections were cut at a thickness ranging from 10-14 microns and for staining, Newton's Iodine-Gentian-violet technique was almost exclusively employed. Since the starch grains also take up the stain, it is necessary to make a sort of a differentiation even at the alcohol stage. Slides had to be kept in 95 per cent. alcohol for a longer period than usual and the cytoplasmic destaining had to be done under microscopical observation. For the study of the glandular hairs small trimmed bits of calyx and other parts of the flower were fixed in F.A.A. and sectioned at a thickness of about 18 microns. These were stained with Heidenhain's iron alum-haematoxylin.

## III. OBSERVATIONS

(a) *Meiosis*.—There are only a few pollen mother-cells in each anther sac. The cells are comparatively big and an interesting feature is the wide disparity in the relative size of the cell and of its nucleus. Sugiura (1936) has pointed out the same peculiarity in *Tecoma Smithii*. The nucleus occupies only a fraction of the total volume of the p.m.c. and is generally towards one side. A number of starch grains of varying sizes are copiously present; Fig. 1 shows a p.m.c. in the first metaphase. It will be seen that the bivalents occupy just the nuclear space. A similar observation was made

by us (Raghavan and Venkatasubban, 1940) in respect of the somatic nuclei of *Alpinia calcarata*.

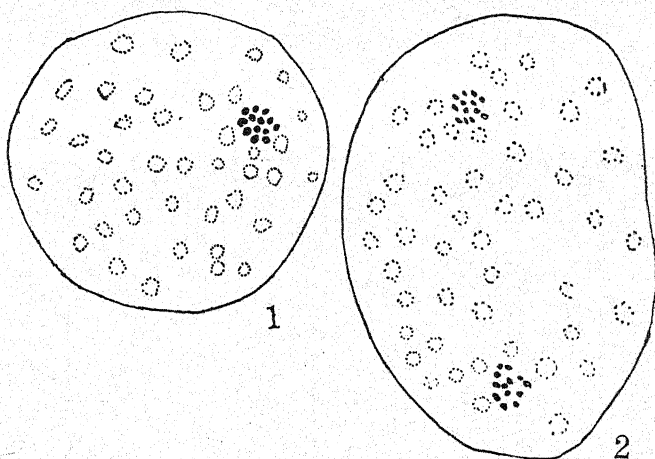


Fig. 1. Pollen mother-cell in the Metaphase I showing 13 bivalents lying to one side and almost filled with starch grains of varying sizes ( $\times$  ca. 3300). Fig. 2. Pollen mother-cell in Metaphase II showing 13/13 distribution ( $\times$  ca. 3300).

Thirteen bivalents could be counted. Meiosis seems to be normal. Anaphasic disjunction follows the usual procedure, there being practically no lagging of chromosomes. Fig. 2 shows the p.m.c. at second metaphase showing the 13/13 distribution. Pollen grains are organised in the usual manner and to all outward appearances are viable. It would appear however that there is something which inhibits seed formation. cursory examinations of the embryo-sacs at different stages have revealed that degenerations are of more frequent occurrence in their formation rather than in the formation of the pollen grains. It looks as if it is the secondary nucleus of the embryo-sac that fails to function. This must however await further detailed observations which are being made.

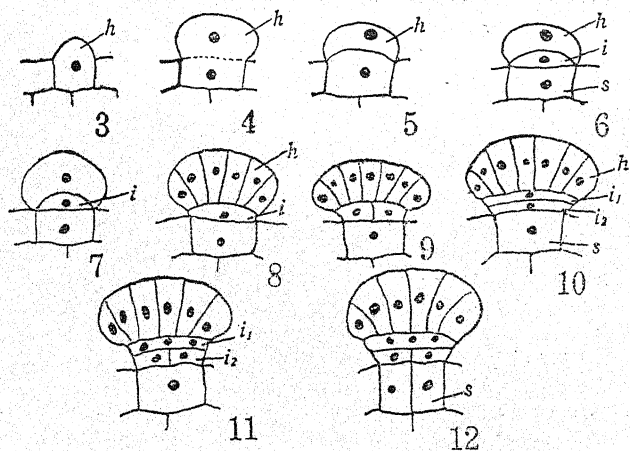
It may be pointed out here that the haploid number 13 has not been met with so far in any other genus belonging to the family Bignoniaceæ. From a casual study of the chromosome numbers, it would seem that aneuploidy has played a large part in the evolution of the species.

(b) *The Secretion*.—The flower buds present an inflated appearance and if a puncture is made the water is ejected with force. It is for this reason that the plant is popularly known as the squirt tree. The average quantity of fluid found secreted within a flower bud, based upon observations on 25 buds of approximately the same stage of development, is 3 ccs. A sufficient quantity of the fluid was not available for a detailed quantitative analysis.

The pH value as determined by the ordinary calorimetric method was found to be 8. The liquid was further tested for the

presence of carbonate, chloride, etc. With HCl it gave an effervescence showing the presence of carbonate. There was also a trace of chloride. The fluid frothed to a small extent and it is likely that it contains a little of saponin too. Test for the presence of tannin was made with ferric chloride but only negative results were obtained. With Fehling's solution there was only a slight colour change and so the presence of only traces of reducing sugars was inferred. On evaporation crystalline bodies of an inorganic nature were left behind, the exact nature of which could not be determined.

(c) *The Trichome*.—The liquid that is collected in such copious quantity is due to the activity of the glandular hairs which form a lining on the inner surface of the calyx. To begin with an epidermal cell throws out a protuberance (Fig. 3, *h*), which becomes gradually widened (Fig. 4, *h*); the nucleus divides into two and a cross wall is formed separating them and this cell (Fig. 5) gradually bulges into the cavity of the knob-like head cell (Fig. 6, *h*). Another tangential wall is laid in such a manner as to separate the basal stalk cell (Fig. 6, *s*) from the head cell (*h*) with the intervention of a small narrow cell (Fig. 6, *i*). In the meantime the nucleus of the stalk cell has divided and the middle cell (Fig. 6, *i* and 7, *i*) comes to possess one of the daughter nuclei of the division. The head cell (Fig. 8, *h*) soon undergoes anticlinal divisions. There are only a number of such anticlinal divisions while periclinal divisions are absent. This indicates that these cells, about 5-7, are uniseriate. The intermediate cell (*i*) undergoes very often another division so that there are usually 2 intermediary cells (Fig. 10, *i*<sub>1</sub> and Fig. 10, *i*<sub>2</sub>); of these the lower one (Fig. 11, *i*<sub>2</sub>) usually undergoes radial septation and forms a two-celled body; the upper (Figs. 11, *i*<sub>1</sub> and 12, *i*<sub>1</sub>) usually becomes 3- or 4-nucleate. Cross wall formation is not quite evident. Occasionally (*i*) undergoes an anticlinal division



Figs. 3-12. Developmental stages of the epidermal hydathodes. Explanation in the text.

(Fig. 9, i). The stalk cell (s) which remains on a level with the rest of the epidermal cells usually remains undivided. But occasionally a radial wall separates it into 2 cells (Fig. 12, s).

#### IV. GENERAL CONSIDERATION

Water-secreting organs or hydathodes vary greatly as regards details of their construction. The physiological importance of this process of water secretion is diversified. Liquid water is secreted when transpiration is suppressed and this prevents the hydrostatic pressure within the conducting system from becoming excessive and this in its turn protects the ventilating system from the danger of flooding.

Of the hydathodes there are two principal types, the epidermal hydathodes which do not communicate directly with the water-conducting system, and secondly those in which the gland is in direct communication with the water-conducting system. They are mostly modified bundle ends at the margins of the leaves. The former corresponds either to a modified epidermal cell or to multicellular trichomes. They are most commonly found to be distributed on the leaves. They range from uni-cellular structures such as found in some Menispermaceæ to multi-cellular water glands such as are plentiful in Plumbaginaceæ. In a large number of cases the epidermal hydathodes take the form of multi-cellular trichomes. In this species they take the shape of shortly stalked capitate hairs. The head which is multi-cellular is responsible for the actual secretion of the fluid. The external cell walls are unthickened and covered by a very thin cuticle. The stalk is to be regarded as the mechanical component of the whole apparatus.

While such hydathodes are common on leaves and as such may be endowed with the function usually ascribed to them, the trichomes found in the calyx in the present case are exactly of the same configuration. Obviously however their functions cannot be identical also. They are to be regarded as modified hydathodes and the large quantity of liquid found secreted enables the corolla and the essential organs of the flower to develop within this fluid without the risk of desiccation. Remembering that this is a tropical African genus such a precaution is very necessary.

The question arises whether these could be called extra-floral nectaries. A nectary is a glandular structure which may be located on any of the various organs, the sugary secretion of which seems to attract pollinating insects to the flowers. A great many plants are furnished with what are called extra-floral nectaries which may occur within the floral region-sepals, bracts or purely on the vegetative parts and they are supposed to serve to attract protective ants, etc. In a species of *Vicia* for example every stipule bears on its upper surface a nectary composed of densely crowded club-shaped hairs. In *Croton sparsiflorus*, a very common exotic weed, two reddish extra floral nectaries are found at the base of the lamina. In all these cases Fehling's test gives a heavy precipitate of cuprous

oxide, a circumstance which indicates the presence of a considerable amount of reducing sugars. In the present case as has been revealed by chemical test, there is indication of only very small traces of sugar. In function the secretion is quite different from ordinary nectariferous secretions. It would therefore appear to be inappropriate to style these as extra-floral nectaries. At best they may be regarded as modified hydathodes.

## V. SUMMARY

1. The haploid chromosome number of *Spathodea campanulata* Beauv. has been determined to be  $n = 13$ .

2. The fluid secreted by the glandular hairs present on the inner side of the calyx has been analysed and is found to contain only traces of reducing sugars.

3. The ontogenetic development of these glandular hairs has been studied in detail.

4. The question how far these glandular structures could be called extra-floral nectaries has been discussed. It is suggested that at best these could be regarded only as modified hydathodes.

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# THE GROWTH OF THE RICE SEEDLINGS (ORYZA SATIVA L., COLUMBA VARIETY, NO. 42) IN SALT SOLUTIONS OF DIFFERENT H-ION CONCENTRATIONS

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(Communicated by R. H. Dastur)

Received for publication on November 18, 1940

THE growth of the rice seedlings in solutions of different salts kept at different pH was determined by Dastur and John (1937) in order to find out the optimum pH and the nature of salts in which maximum growth occurred. The growth of the seedlings was determined by measuring the dry weight of the seedlings before and after they had grown in each solution for a fixed period of time. The pH was varied from 4.0 to 7.0 with a gradation of pH 0.2 for each solution.

As a result of series of experiments, these authors found that the increase in the dry weight of the rice seedlings was greater in solutions of ammonium sulphate and ammonium phosphate than potassium nitrate solution of the same osmotic value and the most favourable pH for the growth was between pH 6.0 and 7.0. All culture solutions of pH below 6.0 were found to be detrimental to the growth of the rice seedlings. They, in their investigation, did not employ culture solutions with pH higher than 7.0 and as they had found that the growth of the rice seedlings was highest at pH 7.0 it would be interesting to find out if a further rise in the pH of the culture solution would bring about an increase or a decrease in the growth of the rice seedlings; the most favourable pH for their growth could thus be determined. Mitra and Phukan (1929) had found that the highest root development occurred at pH 7.9. They only took the length of the roots as a measure of growth. In order to have a correct measure of growth, it is necessary to obtain the dry weight of the whole seedlings. Therefore the authors undertook to measure by the dry weight method the growth of the rice seedlings in salt solutions of a pH varying from pH 6.0 to pH 8.0.

## INVESTIGATION

In these experiments the range of pH used was from 6.0 to 8.0 which means approximately pH 1.0 on each side of the neutral point. The gradation of pH was varied by 0.2 thus using solutions of eleven different pH for each salt solution. Salts used were sulphates of Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup> and Mg<sup>++</sup>, nitrates of Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Ca<sup>++</sup>



and  $Mg^{++}$ , and phosphates of  $Na^+$ ,  $K^+$  and  $NH_4^+$ . In addition to these salts Knop's and Tottingham's culture solutions were also used. Previous workers had observed that the number of plants fixed in each culture jar had a marked effect upon the concentration and pH of the culture solution. When a larger number of plants were used the concentration and pH of the solution changed very rapidly. In all these experiments, therefore, the number of plants used was only 12. The pH of each solution was kept the same during the period of experiment by adding alkali or acid to the solution as required from day to day. The different salts used were added in such quantities, that the solutions obtained were nearly iso-osmotic. The change in the pH value of the solutions was noted every day and the solutions were changed twice a week. The other methods were the same as those described by Dastur and John (1937).

#### DETERMINATION OF H-ION CONCENTRATION

*Colorimetric method.*—H-ion concentration of culture and various salt solutions were determined by the colorimetric method as given by Clark (1926), but this method was abandoned later, since there were errors in technique and certain specific errors such as salt errors, the indicator error and others which rendered it unsuitable for investigations requiring accuracy. On account of this, the electrometric method was used in all the experiments with the exception of those cases in which pH was above 7.8 where colorimetric method had to be used.

#### EXPERIMENTAL METHOD

Rice seeds of Columba variety, No. 42, were soaked in water for 24 hours. These seeds after they had germinated were planted in saw dust and allowed to grow for 14 days. On the 15th day they were taken out *en bloc* so that the roots were not injured. The seed coat together with endosperm of each seedling were then removed. About 120 seedlings of equal growth and apparently similar condition of health were selected for experiments. Special culture jars of 1500 c.c. capacity were chosen and fitted with wooden lids having about seven holes in all for experimentation. These were then filled with distilled water and required amount of the stock culture solution or salt solutions of normal concentrations was added to it. There were three repetitions of each series and the mean of the three replicates is given in Table I.

A study of the figures in the table shows that maximum increase in dry weight of seedlings occurred at pH 7.0 to pH 7.2 in all salts irrespective of the kations. Growth was maximum in potassium nitrate and ammonium nitrate solutions. The minimum increase in dry weights of the seedlings was found to occur in calcium nitrate solution.

These results were put to statistical test by employing Fisher's (1937) method of analysis of variance. It is clear that the effects

TABLE I

*Increase in dry weight, in mgs. of the rice seedlings over the initial weight of the seedlings in different nitrate solutions at different pH*

(Mean of the three replicates)

pH	Calcium nitrate	Magnesium nitrate	Sodium nitrate	Potassium nitrate	Ammonium nitrate	pH mean under all kations
6.0	1.9	5.3	2.6	5.5	9.6	5.0
6.2	2.1	6.8	10.7	9.5	14.4	8.7
6.4	3.0	7.0	10.7	11.4	20.1	10.4
6.6	5.4	14.1	16.3	15.7	25.9	15.5
6.8	8.1	14.2	18.3	15.7	26.3	16.6
7.0	9.6	15.1	18.8	20.4	36.9	20.1
7.2	11.4	17.2	20.3	33.9	21.2	20.8
7.4	8.4	8.3	9.3	23.2	14.1	12.5
7.6	6.4	7.0	6.6	13.8	6.6	8.1
7.8	5.4	4.9	6.7	9.6	5.9	6.5
8.0	3.4	4.5	3.3	9.3	2.9	4.7
Kation mean under all pH	5.9	9.5	11.3	15.3	16.7	11.7

C.D. (1%) for kations mean under all pH = 1.59.

C.D. (1%) for pH mean under all kations = 2.39.

C.D. (1%) for pH  $\times$  kation mean for body of the table = 5.36.

on growth of the rice seedlings of kations and of pH are highly significant indicating that different kations or different pH have produced different effects on the growth of the seedlings. The effect of one kation on growth is significantly different from the effect of another kation. Thus the effect of potassium nitrate on the growth of the rice seedlings is significantly different from the effect of any other nitrate. The same remarks apply to the different pH of the salt solutions.

The interaction between kations and pH is highly significant also. It shows that the effect on growth of a particular kation is

modified by the pH of the salt solution, *i.e.*, it is different for the same salt solution at different pH.

*Analysis of Variance*

Variance due to	D.F.	Sum of squares	Mean squares	Ratio of variance
Kations .. ..	4	2538.70	634.7	100.09
pH .. ..	10	5057.81	505.8	79.77
Interaction— kations $\times$ pH .. ..	40	2288.59	57.21	9.02
Residual error .. ..	110	697.54	6.341	
TOTAL .. ..	164	10582.64		

*The effect of Kations and pH in different nitrate salts on dry weights.*—With  $K^+$ , pH 7.2 gave maximum weight and with  $NH_4^+$ , pH 7.0 gave maximum weight. Both these were equal to each other and represented the maximum weights obtained in the experiments. The dry weights for pH 7.2 and 7.0 did not differ significantly from the other kations and between them  $Ca^{++}$  was the lowest at both pH values (Table I).

The next best weights were obtained at pH 6.8 with  $NH_4^+$ ; not significantly differing from it was the weight for  $K^+$ , at pH 7.4, these were midway between those at pH 7.2 and 7.0 with the same ions respectively. The other ions made no difference for pH 7.4. At pH 6.8 the order for the other ions was  $Na^+$ ,  $Mg^{++}$ , and  $Ca^{++}$ ; for pH 6.6  $Na^+ = Mg^{++}$  and  $Ca^{++}$ .

Other differences in the effects on dry weights of the plant can be read off directly from Table I.

Here the maximum increase in the dry weight occurred at pH 7.2 in three out of the four sulphate solutions used. It is remarkable to find that the maximum growth occurred in solution of magnesium sulphate. It is difficult to explain such a large increase in dry weight of seedlings in the solution of magnesium sulphate. The growth of the seedlings in this solution was found to be greater than in ammonium sulphate solution.

*—The effect of Kations and pH in different sulphate salts on dry weight.*—With  $Mg^{++}$ , pH 7.2 gave maximum increase of weight and with  $NH_4^+$  pH 7.0 was the best (Table II). With  $Na^+$ , increase in weight at pH 7.0 and 7.2 was highest and did not vary significantly from each other. While with  $K^+$ , pH 7.2 was the best. The maximum growth obtained in  $SO_4^{--}$  series was with  $Mg^{++}$ , the next best in order being  $NH_4^+$ ,  $Na^+$  and  $K^+$ . The growth increase in  $Mg^{++}$  at

TABLE II

*Increase in dry weight in mgs. of the rice seedlings over the initial weights of the seedlings in different sulphate solutions at different pH*

(Mean of the three replicates)

pH	K <sub>2</sub> SO <sub>4</sub> "	Na <sub>2</sub> SO <sub>4</sub> "	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> "	Mg <sup>++</sup> SO <sub>4</sub> "	pH mean under all kations
6.0	1.0	2.4	7.6	5.5	4.1
6.2	1.9	7.5	7.7	2.2	4.8
6.4	4.6	7.9	9.2	11.0	8.2
6.6	6.9	10.4	14.8	13.1	11.3
6.8	9.1	12.7	16.5	15.4	13.4
7.0	10.4	14.8	20.0	23.2	17.1
7.2	13.4	17.0	15.9	31.0	19.4
7.4	9.7	14.0	8.3	17.5	12.4
7.6	8.4	10.2	8.0	14.0	10.2
7.8	6.6	8.0	6.1	7.8	7.1
8.0	3.6	4.8	4.4	6.9	4.9
Kation mean under all pH	6.9	10.0	10.8	13.4	10.26

C.D. (1%) for kations mean under all pH = 2.28.

C.D. (1%) for pH mean under all kations = 3.81.

C.D. (1%) for pH × kations in the body of the table = 8.01.

pH 7.0 and 7.4 lay in the same non-significant group, with the maximum of NH<sub>4</sub><sup>+</sup> at pH 7.0; and increase of weight in Mg<sup>++</sup>, at pH 6.8 and 7.4 lay in the same non-significant group with the highest growth with Na<sup>+</sup> at pH 7.0 and 7.2 which was superior to the best growth obtained in K<sup>+</sup> at pH 7.2. The next best growth was at pH 7.0 with NH<sub>4</sub><sup>+</sup>, from which the growth obtained in Mg<sup>++</sup> at pH 7.4 did not vary significantly. The other ions at pH 7.4 did not make any difference of grouping. The next best pH was 6.6, 6.8 and 7.4. At pH 6.6, the order for the ions was NH<sub>4</sub><sup>+</sup>, Mg<sup>++</sup>, Na<sup>+</sup>, K<sup>+</sup>, and at pH 6.8 it was Mg<sup>++</sup> = NH<sub>4</sub><sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup> and at pH 7.4 it was Mg<sup>++</sup> = Na<sup>+</sup>, K<sup>+</sup> and NH<sub>4</sub><sup>+</sup>. So the order instead of being Mg<sup>++</sup>, NH<sub>4</sub><sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup> at pH

6.6  $\text{NH}_4^+$  is superior to  $\text{Mg}^{++}$ , while  $\text{Na}^+$  and  $\text{K}^+$  are below that. But at pH 6.8  $\text{Mg}^{++}$  and  $\text{NH}_4^+$  again become equal which means that pH above 6.6 is suitable for  $\text{Mg}^{++}$ .

### Analysis of Variance

Variance due to			D.F.	Sum of squares	Mean squares	Ratio of variance
Kations	..	..	3	723.37	241.12	18.46**
pH	..	..	10	3046.50	304.65	23.32**
Interaction— kations $\times$ pH	..	..	30	833.75	27.79	2.12**
Residual error	..	..	88	1149.46	13.06	
TOTAL			131	5753.08		

*Effect of different Kations and pH in phosphate salts on dry weights.*—With  $\text{K}^+$  at pH 7.0 and 7.2, and  $\text{NH}_4^+$  at pH 7.2 the highest increase in growth was obtained. While with  $\text{Na}^+$ , pH 7.0 was the best (Table III). The highest growth obtained in the phosphate series was with  $\text{NH}_4^+$  at pH 7.2, though it did not vary significantly from  $\text{K}^+$  at pH 7.0 and 7.2. The next best growth in  $\text{NH}_4^+$  was at pH 7.0 and 7.4 which lay in the same non-significant group. But increase in weight with  $\text{K}^+$  at pH 7.0 was greater than that with  $\text{NH}_4^+$  at pH 7.0, while at pH 7.4  $\text{NH}_4^+$  is greater than  $\text{K}^+$ . It means that under the influence of ammonium phosphate pH higher than pH 7.0 was still suitable for growth.

The next best growth amongst phosphates was at pH 6.8 and 6.6. At this stage the order of ions was  $\text{K}^+$ ,  $\text{NH}_4^+$  and  $\text{Na}^+$ , though they lay in the same non-significant groups. The next best pH was 6.4. At this stage the order of the ions was  $\text{K}^+$ ,  $\text{NH}_4^+$  and  $\text{Na}^+$ .  $\text{K}^+$  at pH 6.4 was greater than  $\text{NH}_4^+$  though the significant difference between them was not great. But  $\text{K}^+$  and  $\text{NH}_4^+$  were significantly superior to  $\text{Na}^+$ . The influence of  $\text{Na}^+$  had brought down the value of pH 7.0 to equality with pH 6.6 and 6.8 for  $\text{K}^+$  and  $\text{NH}_4^+$ . The next best growth was at pH 6.4. But here  $\text{K}^+$  is greater than  $\text{NH}_4^+$  while  $\text{K}^+$  and  $\text{NH}_4^+$  were significantly superior to  $\text{Na}^+$ . Next best pH was 6.2 and 6.0. At this stage the order was  $\text{NH}_4^+ = \text{K}^+$ , and  $\text{Na}^+$ . The values of increase in weight at pH 6.0 and 6.2 were superior in all cases to the values of increase at pH 7.8 and 8.0. But in  $\text{NH}_4^+$  and  $\text{K}^+$  the values lay in the same non-significant group. Least growth was noticed at pH 8.0. Of all the salts of the phosphate series growth was significantly less in  $\text{Na}^+$  while  $\text{NH}_4^+$  though better than  $\text{K}^+$  lay in the same non-significant group.

TABLE III

*Increase in dry weight in mgs. of the rice seedlings over the initial weights of the seedlings in different phosphate solutions at different pH*

(Mean of the three replicates)

pH	Sodium phosphate	Potassium phosphate	Ammonium phosphate	pH mean under all kations
6.0	6.4	6.2	14.1	8.9
6.2	7.2	12.7	14.3	11.7
6.4	9.1	20.3	17.4	15.6
6.6	13.4	21.3	18.3	17.7
6.8	16.2	24.2	18.7	19.7
7.0	18.5	21.0	28.5	26.0
7.2	15.8	29.0	22.5	25.8
7.4	15.2	24.7	28.7	22.9
7.6	6.0	14.3	27.6	16.0
7.8	3.8	12.2	11.3	9.1
8.0	3.1	12.0	8.1	7.7
Kation mean under all pH	10.4	19.0	19.9	16.4

C.D. (1%) for kations mean under all pH = 1.23.

C.D. (1%) for pH mean under all kations = 2.40.

C.D. (1%) for pH × kations mean for the body of the table = 4.06.

The greatest increase in dry weight occurred in the ammonium and potassium phosphate solutions.

#### *Analysis of Variance*

Variance due to	D.F.	Sum of squares	Mean squares	Ratio of variance
Kations .. ..	2	1821.68	910.84	241.60
pH .. ..	10	3960.54	396.05	105.06
Interaction— kations × pH .. ..	20	787.09	39.35	10.42
Residual error .. ..	66	249.34	3.77	
TOTAL .. ..	98	6818.65		

TABLE IV

*Increase in dry weight in mgs. of the rice seedlings over the initial weights of the seedlings in Knop's and Tottingham's culture solutions at different pH.*

(Mean of the three replicates)

pH	Knop's culture solution	Tottingham's culture solution	pH mean under the two culture solutions
6.0	11.3	8.8	10.05
6.2	9.6	9.1	9.35
6.4	8.8	9.4	8.6
6.6	13.6	13.2	13.4
6.8	19.0	20.6	19.8
7.0	24.2	22.0	23.1
7.2	26.5	23.7	25.1
7.4	15.2	20.6	17.9
7.6	12.8	17.7	15.25
7.8	11.8	9.6	10.7
8.0	10.0	9.1	9.55
Culture solution mean under all pH	14.8	14.8	14.8

Here also the most favourable reaction for growth was pH 7.2. There was no differential effect on growth of the two culture solutions.

The results show conclusively that pH in the neighbourhood of pH 7 and 7.2 is best for growth of these rice seedlings while growth is decreased at pH higher or lower than this value.

The average increase in the dry weights of the rice seedlings after they had grown in the two culture solutions and in solutions of nitrates, sulphates and phosphates at different pH are represented in Figs. 1 to 4.



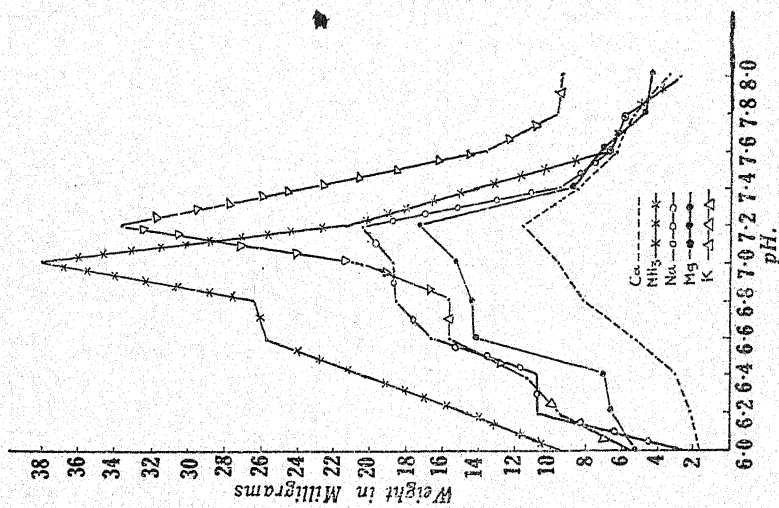


FIG. 1  
Nitrate Series

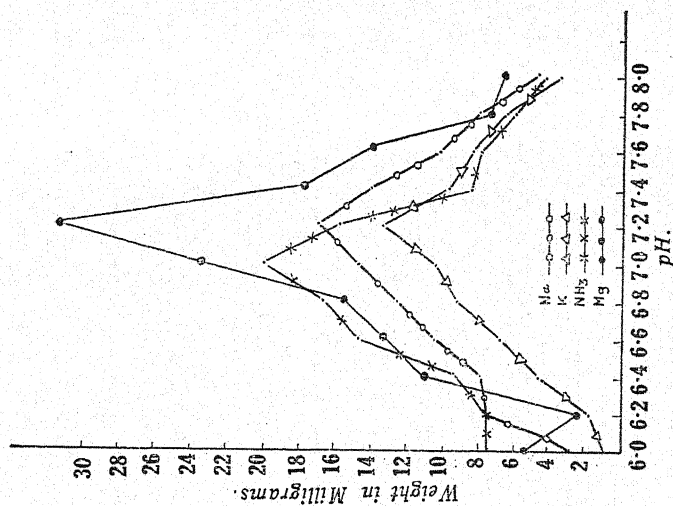


FIG. 2  
Sulphate Series



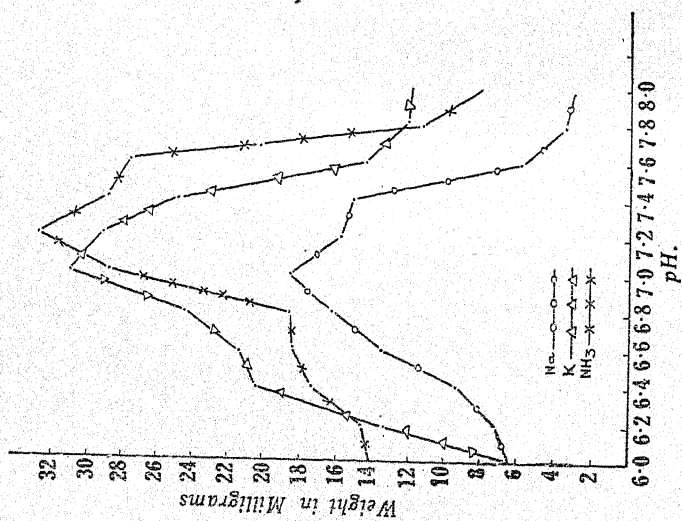


FIG. 3  
Phosphate Series

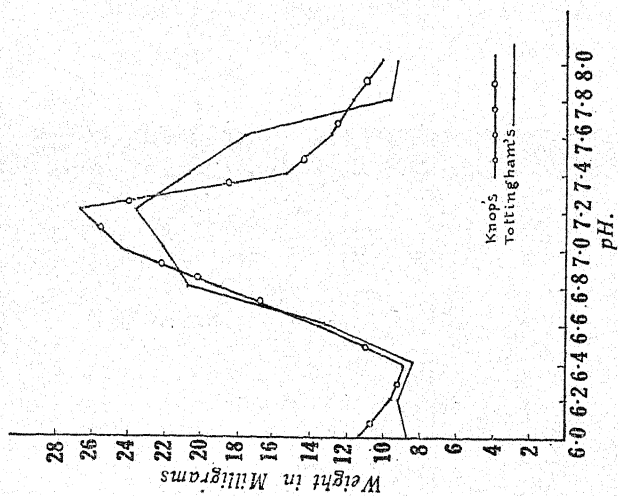


FIG. 4  
Culture Solutions

## CONCLUSIONS

The important conclusions reached as a result of this investigation are summarised below :

1. The most favourable reaction for the growth of the rice seedlings was found at pH 7 and 7.2 for all salts and culture solutions.

2. The maximum increase in dry weights amongst the nitrates was obtained in the ammonium nitrate and potassium nitrate solutions the two salts being equal in their effects.

3. The minimum increase in dry weight was obtained in the calcium nitrate solutions.

4. The maximum increase in dry weight was obtained in ammonium nitrate solution at pH 7.0, while in the potassium nitrate solution it was at pH 7.2.

5. The maximum increase in dry weight of the rice seedlings amongst the phosphates was obtained in ammonium phosphate and potassium phosphate solutions, both being equal in their effects.

6. The maximum increase in dry weights of the rice seedlings amongst the sulphates was obtained in the magnesium sulphate solution at pH 7.2.

7. In general phosphates were found superior to nitrates and sulphates, as the greatest increase in dry weights occurred in the phosphate series. This is probably due to very small growth made by the seedlings in calcium and magnesium nitrate. If these two are not taken into account, the nitrates of ammonium and potassium are superior to phosphates of the same two kations. But sodium phosphate on the other hand has given greater increase in dry weight than sodium nitrate.

8. There is a greater fall in dry weights in the nitrates than in the phosphates of different kations as the pH of the solution increases from pH 7.2 onwards. If the solutions of all the pH values are considered together, the average increase in the dry weights in the ammonium and potassium nitrate solutions are in lower than in the phosphates of the same kations. Thus for the rice seedlings the range of favourable reaction is wider for the phosphates than for the nitrates.

9. The superiority of magnesium sulphate for the growth of the rice seedlings over the sulphates of the potassium and ammonium has been wholly unexpected and it is difficult to explain it at present.

10. The interaction of kations with pH was highly significant, indicating that the effect of each kation on growth was modified by the pH of the salt solutions.

## SUMMARY

The study of the relative effects of the solutions of nitrates, sulphates and phosphates of different bases on the growth of the rice seedlings at different H-ion concentrations varying from pH 6.0 to 8.0 was made and it was found that the maximum growth

of the rice seedlings occurred in all salt solutions with pH 7.0 or pH 7.2. In solutions of ammonium salts and phosphates of other salts the maximum growth was at pH 7.0, while in the rest of the salt solutions and in the Knop's and Totttingham's culture solutions the maximum growth was at pH 7.2. The favourable pH for the growth of the rice seedlings was between pH 6.8 and 7.2. The growth was markedly suppressed at pH lower or higher than this range.

Growth of the rice seedlings was the highest in the nitrates, medium in phosphates and least in sulphates. Amongst the nitrates, potassium and ammonium nitrates were found to be the best, while the least growth occurred in solutions of nitrates of calcium and magnesium. Similarly phosphates of ammonium and potassium were superior to sodium phosphate. Amongst sulphates, magnesium sulphate was found to be superior to sulphates of ammonium and potassium.

The growth data was statistically examined by Fisher's method of analysis of variance. It was found that the effect of different kations and different pH on growth of the rice seedlings was highly significant, indicating that each kation and each pH had a different effect on the growth and the effect of each kation on growth was modified by the pH of the solution.

#### ACKNOWLEDGEMENT

The authors' best thanks are due to Prof. R. H. Dastur, M.Sc., F.L.S., for his criticism and valuable help during the course of this investigation.

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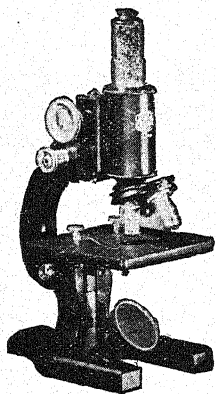
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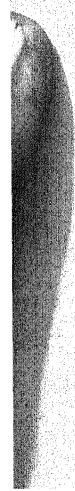
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# Contents of this Number

	PAGE
Chatterjee, D. Two New Anonaceæ from Assam and Burma	1
Krishna Iyengar, C. V. Development of Embryo-sac and Endosperm-Haustoria in some Members of Scrophulari- neæ. V. <i>Ilysanthes hyssopioides</i> Benth., <i>Bonnaya tenuifolia</i> Spreng. . . . .	5
Shri Ranjan. Studies on the Photochemical Action in Plants. I. The respiration of entire <i>Pistia</i> plants in light . .	19
Anantaswamy Rau, M. Studies in the Apocynaceæ . .	33
Tatachar, T. The Development of the Embryo-sac and Formation of Haustoria in <i>Lantana indica</i> Roxb., and <i>Stachytarpheta indica</i> Vahl . . . . .	45
Raghava Rao, K. V. Gametogenesis and Embryogeny in Five Species of the Convolvulaceæ . . . . .	53
Thirumalachar, M. J. A Method for Germinating and Stain- ing Teleutospores . . . . .	71
Chatterjee, D., and Mukerjee, S. K. Some New Plants from India and Burma . . . . .	77
Shri Ranjan. Studies on the Photochemical Action in Plants. II. Photosynthesis in leaves at different temperatures . .	91
Shri Ranjan and Brij Behari Lal Saksena. Studies on the Photochemical Action in Plants. III. The Influence of visible light on the rate of respiration of some coloured flowers . . . . .	99
Shri Ranjan. Studies on the Photochemical Action in Plants. IV. The effect of violet and ultra-violet radiations on plant respiration . . . . .	105
Doraiswami, S. On the Morphology and Cytology of <i>Eudorina indica</i> Iyengar . . . . .	113
Biswas, K. Systematic Position of a little known Flowering Plant from South Burma . . . . .	141
Sen, P. Effect of Anthocyanin Pigments on the Rate of Photosynthesis in <i>Eranthemum</i> Spp. . . . .	147
Iyengar, M. O. P., and Kanthamma, S. On <i>Hormidiella</i> , A New Member of the Ulotrichaceæ . . . . .	157
Iyengar, M. O. P., and Kanthamma, S. A Note on <i>Ulotrichopsis viridis</i> gen. et sp. nov. . . . .	167
Kanthamma, S. On the Life-History of <i>Characium</i> <i>terrestris</i> sp. nov. . . . .	171
Iyengar, M. O. P., and Ramanathan, K. R. On the Repro- duction of <i>Anadyomene stellata</i> (Wulf.) Ag. (Preliminary Note) . . . . .	175
Book Review. Indian Labiata . . . . .	177

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## Contents of this Number

---

	PAGE
Banerji, I. A Contribution to the Life-History of <i>Costus speciosus</i> Smith .. .. .	181
Srinivasan, V. K. Morphological and Cytological Studies in the Scrophulariaceæ. II. Floral Morphology and Embryology of <i>Angelonia grandiflora</i> C. Morr. and related genera .. .. .	197
Parija, P., and Mallik, P. Nature of the Reserve Food in Seeds and Their Resistance to High Temperature ..	223
Ekambaram, T., and Kamalam, V. K. Permeability of the Wall of the Xylem Vessel .. .. .	231

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## Contents of this Number

---

	PAGE
Mullan, D. P. The root-structure of <i>Chlorophytum tuberosum</i> Baker .. .. .	235
Iyengar, M. O. P., and Ramanathan, K. R. <i>Cladospongia</i> , a new member of the Craspedomonadaceae from Madras .. .. .	241
Randhawa, M. S. <i>Zygogonium kumaoensis</i> , a new species of <i>Zygogonium</i> from Kumaon .. .. .	247
Krishna Iyengar, C. V. Structure and development of seed in <i>Sopubia trifida</i> Ham. .. .. .	251
Singh, B. N., and Mehra, S. N. The significance of anatomical changes accompanying regeneration of X-rayed <i>Bryophyllum</i> leaves .. .. .	263
Sundar Rao, Y. Structure and development of the embryo-sac of <i>Drimiopsis kirki</i> Baker and <i>Allium govanianum</i> Wall. .. .. .	273
Raghavan, T. S., and Srinivasan, V. K. A contribution to the life-history of <i>Bergia capensis</i> Linn. .. .. .	283
Raghavan, T. S., and Venkatasubban, K. R. Studies in the Bignoniaceæ. I. Chromosome Number and Epidermal Hydathodes in <i>Spathodea campanulata</i> Beauv. ..	293
Cooper, R. E., and Sohoney, D. V. The growth of the Rice Seedlings ( <i>Oryza sativa</i> L., Columba variety, No. 42) in salt solutions of different H-ion concentrations ..	299